Lowest numbers of primary CD8§ T cells can reconstitute protective immunity upon adoptive immunotherapy

Christian Stemberger,1,2 Patricia Graef,1 Marcus Odendahl,3 Julia Albrecht,4 Georg Dössinger,1 Florian Anderl,1 Veit R. Buchholz,1 Georg Gasteiger,5,6 Matthias Schiemann,1,5 Götz U. Grigoleit,7 Friedhelm R. Schuster,8 Arndt Borkhardt,8 Birgitta Verslues,9 Torsten Tonn,3,10 Erhard Seifried,11 Hermann Einsele,7 Lothar Germeroth,12 Dirk H. Busch,1,2,4,5,13 and Michael Neuenhahn1,4,5,13

1Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany; 2Focus Group “Clinical Cell Processing and Purification,” Institute for Advanced Study, Technische Universität München, Munich, Germany; 3Institute for Transfusion Medicine, German Red Cross Blood Donation Service North-East, Dresden, Germany; 4Clinical Cooperation Group “Immune Monitoring,” and 5Clinical Cooperation Group “Antigen-specific Immunotherapy,” Heimholz Center Munich (Neuherberg) and Technische Universität München, Munich, Germany; 6Institute for Virology, Technische Universität München and Helmholtz Zentrum München, Munich, Germany; 7Department of Internal Medicine II, University of Würzburg, Würzburg, Germany; 8Clinic of Paediatric Oncology, Haematology and Clinical Immunology, Centre for Child and Adolescent Health, Medical Faculty, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; 9Paediatric Blood and Marrow Transplantation Program, University Medical Center Utrecht (Wilhelmina Children’s Hospital), Utrecht, The Netherlands; 10Institute of Immunology, Medical Faculty, Dresden University of Technology, Dresden and Center for Regenerative Therapies Dresden, Dresden, Germany; 11Institute for Transfusion Medicine and Immunohematology, German Red Cross Blood Donor Service, Johann Wolfgang Goethe University, Frankfurt, Germany; 12Stage Cell Therapeutics, Göttingen, Germany; and 13German Center for Infection Research, Munich, Germany

Introduction

After allogeneic hematopoietic stem cell transplantation (allo-HSCT), severe impairment of the patient’s T-cell compartment due to lymphocyte-depleting conditioning regimens regularly leads to reactivation of highly prevalent endogenous herpes viruses like Epstein-Barr virus, herpes simplex virus, varicella zoster virus, or cytomegalovirus (CMV). In particular, CMV can contribute substantially to direct and indirect infection-related complications in allo-HSCT patients if donor-derived virus-specific T cells cannot timely control virus replication.1,2 Prophylactic or preemptive virostatic treatment with ganciclovir or foscarinet is known to be effective but exhibits substantial side effects.3 Therefore, adoptive transfer of donor-derived virus-specific T cells has been proposed as an alternative treatment option in order to restore antiviral immunity and bridge the first months of high susceptibility after allo-HSCT.

Pilot studies in the early 1990s have convincingly illustrated the efficacy of this approach,4-7 which has further been adapted to target a wide range of infectious and noninfectious complications.8,9 Yet, the introduction of antiviral adoptive T-cell transfer into routine treatment after allo-HSCT has so far been discouraged by costly and time-consuming Good Manufacturing Practices–conform in vitro expansions.10

More recently, direct ex vivo isolation (<24h) of virus-specific T cells using conventional11 or minimally manipulating reversible
major histocompatibility complex (MHC) multimers\textsuperscript{12,13} as well as short-time–stimulated cytokine-secreting T cells\textsuperscript{14-16} has been successfully tested in clinical pilot studies.

However, yield of these primary virus-specific T cells can be limited by cell isolation efficiency from small antigen-specific donor T-cell populations. In addition, the content of contaminating, potentially graft-versus-host disease (GVHD)-triggering CD3\textsuperscript{+} T cells restricts the total number of adoptively transferred T cells.\textsuperscript{17} In particular, for the envisioned prophylactic strategies or the recently proposed use of partially HLA-matched third-party donors,\textsuperscript{18-20} the use of small-sized clinical T-cell products might become indispensable to keep the risk of GVHD as low as possible.

Because the minimal number of ex vivo–isolated cells for successful T-cell therapy is unknown, we decided to test the potential of minimal numbers of ex vivo–isolated antigen-specific T cells in a well-established murine infection model with the intracellular bacterium \textit{Listeria monocytogenes} (\textit{L.m.}). After systemic application in mice, \textit{L.m.} uses cellular niches to survive initially in the spleen.\textsuperscript{21,22} Although infection is primarily confined by innate defense mechanisms, clearance of \textit{L.m.} depends on the mobilization of adaptive immunity, illustrated by chronic \textit{L.m.} infection in severe combined immunodeficiency (SCID) mice.\textsuperscript{23} The established (eventually) lifelong T-cell immunity is mainly mediated by antigen-experienced CD8\textsuperscript{+} memory T cells, and contribution of different memory subsets to protective T-cell responses has been controversially discussed during the last decade. However, in the context of adoptive T-cell transfer, data are accumulating that less differentiated memory subsets (eg, CD62L\textsuperscript{hi} cells) might comprehend all necessary qualities for in vivo efficacy, in particular if implemented for prophylactic use: long-term survival, extensive proliferative capacity, and differentiation potential into effector and effector memory cells that finally convey cytotoxic control.\textsuperscript{24-27}

In addition, we could recently show that single naive \textit{L.m.}-epitope–specific CD8\textsuperscript{+} T cells can differentiate into diverse effector and memory T-cell subsets.\textsuperscript{28,29} Here, we used a comparable single-cell transfer protocol to evaluate the protective capacity of minimal numbers of naive \textit{L.m.}-epitope–specific CD8\textsuperscript{+} T cells after in vivo challenge and in addition extended these analyses to CD62L\textsuperscript{hi} and CD62L\textsuperscript{lo} CD8\textsuperscript{+} memory T cells. The lowest numbers of CD62L\textsuperscript{hi} memory T cells developed into diversified progenies conferring protection against \textit{L.m.} challenge, identifying this subpopulation as the most potent for effective adoptive immunotherapy. Finally, the reconstitution capacity of human low-dose T-cell transfers was demonstrated by the expansion of Streptamer-enriched CMV-specific CD8\textsuperscript{+} T cells in 2 compassionate-use allo-HSCT patients.

**Methods**

**Mice and \textit{L.m.} infection**

CD45.2\textsuperscript{+} C57BL/6 wild-type (B6 wt) mice were obtained from H. Winkelmann (Borchen, Germany). CD45.1\textsuperscript{+} congenic C57BL/6 (CD45.1) and CD45.2\textsuperscript{+} RAG1-deficient (RAG\textsuperscript{−/−}) mice and CD45.1\textsuperscript{+} K\textsuperscript{b}-ovalbumin (Ova\textsubscript{257-264}) peptide–specific T-cell receptor (TCR) C57BL/6 transgenic mice (CD45.1-OT-I) were derived from in-house breeding. Experimental conditions of adoptive transfer and \textit{L.m.} infection experiments are provided in the supplemental Methods (available at the \textit{Blood} Web site).

**Isolation of ova-specific donor T cells**

Naive CD45.1-OT-I T cells, antigen-experienced CD45.1\textsuperscript{+} OT-I memory T cells, or polyclonal Ova\textsubscript{257-264}Peptide–specific CD45.1\textsuperscript{+} memory T cells were used for adoptive cell transfer. See supplemental Methods for details.

**Cell sorting and adoptive transfer of T cells**

The adoptive cell transfer of 1 to 1000 antigen-specific CD8\textsuperscript{+} T cells has been previously described\textsuperscript{30} and is described in detail in the supplemental Methods.

**MVA-Ova immunization and \textit{L.m.-Ova} challenge in recipient mice**

Recipient mice were prime-boost immunized by IV injection with 2 subsequent doses (1 \times 10\textsuperscript{5} colony-forming units [CFUs]) of a replication-deficient modified vaccinia virus type Ankara recombinantly expressing ova under control of the viral P7.5 promoter (MVA-Ova).\textsuperscript{31} Expansion and differentiation of T-cell progenies were followed by fluorescence-activated cell sorter (FACS) staining of blood and ex vivo tissue samples as previously described,\textsuperscript{32} and protective capacity of donor-derived T-cell responses was tested in adoptively transferred T-cell–deficient RAG\textsuperscript{−/−} recipient mice by \textit{L.m.-Ova} infection. See supplemental Methods for further details.

**Patients**

Two patients were treated with allo-HSCT for SCID syndrome and B-cell acute lymphoblastic leukemia (B-ALL), respectively. Patients suffered before and/or after stem cell transplantation from a therapy-resistant CMV viremia.

**Isolation of human CMV-specific donor lymphocytes**

CMV–specific CD8\textsuperscript{+} T cells were purified from stem cell donor–derived peripheral blood mononuclear cells (PBMCs) using HLA-Streptamers as previously described.\textsuperscript{12,13} See supplemental Methods for further details.

**Tracking of donor-derived CMV HLA-A0201/pp65–specific CD8\textsuperscript{+} T cells**

CDR3 sequencing of ex vivo–isolated transferred T cells allowed identification of donor-derived T cells as previously described.\textsuperscript{13} See supplemental Methods for details.

Approval for the transplantation and the compassionate use treatment was obtained from the Medical Ethical Board of the University Medical Center Utrecht and the Medical Faculty Ethics Committee of Heinrich-Heine University Düsseldorf, respectively. Informed consent was provided according to the Declaration of Helsinki.

**Results**

**Low-dose transfer of naive ova-peptide–specific T cells confers protection against \textit{L.m.-Ova} challenge**

In murine \textit{L.m.-Ova} infection, single adoptively transferred ovalbumin–peptide–specific CD8\textsuperscript{+} T cells can give rise to highly diversified T-cell populations. Those progenies can consist of both effector and memory T cells and resemble herein concomitantly developing endogenous T-cell responses in B6 wt hosts.\textsuperscript{28,29} However, whether developing T cells from such lowest-cell-dose transfers will also be sufficient to protect against full-scale infection has not yet been determined. To address this question in regard to its clinical relevance, we used T- and B-cell–devoid RAG\textsuperscript{−/−} recipient mice,\textsuperscript{32} in which any functional antibacterial T-cell response could be unambiguously attributed to the progeny of adoptively transferred T cells. \textit{L.m.-} infected T- and B-cell–deficient mice are not able to eradicate the pathogen, and chronic infection develops.\textsuperscript{23} In order to study the expansion potential as well as the protective capacity of low-dose adoptive T-cell transfers in immunocompromised hosts, we used MVA-Ova for prime-boost vaccination prior to challenge with \textit{L.m.-Ova}. RAG\textsuperscript{−/−} mice received a first MVA-Ova dose briefly after adoptive T-cell transfer followed by a boost vaccination.

From [www.bloodjournal.org](http://www.bloodjournal.org) by guest on April 7, 2017. For personal use only.
14 days later (Figure 1A). Expansion of transferred CD45.1+ CD8+ T cells was subsequently followed in peripheral blood. In accordance with our previously published data,28,29 transfer of 100 CD45.1+ OT-1 T cells was found to be successful in all recipient mice, and single-cell transfers still resulted in detectable antigen-specific T-cell populations in peripheral blood of 15% to 20% of recipients (data not shown).28,29 After challenge with an otherwise- lethal dose of L.m.-Ova, all successfully single-cell- transferred mice had no detectable bacteria in liver and spleen, whereas bacterial loads were at least 100- to 1000-fold higher in recipients with no detectable T cells after single-cell transfer and vaccination (Figure 1B). Taken together, these data show that even the lowest amounts of adoptively transferred naive antigen-specific CD8+ T cells (and, in the extreme, even a single cell) can establish a functional T-cell response in RAG−/− hosts leading to complete protection against high-dose bacterial challenge.

Next, we tested if the lowest numbers of transferred naive antigen-specific CD8+ T cells can directly contain bacterial growth in a preemptive setting in RAG−/− mice without previous MVA-Ova vaccination. Mice were infected with a sublethal dose of L.m.-Ova immediately after transfer of naive CD45.1+ OT-1 cells, and bacterial replication was determined by CFU counts in the spleen and liver 9 days later (Figure 1A). As previously described for L.m.-infected SCID mice,23,34 high bacterial numbers (mean 10^5 CFUs; Figure 1C) were counted in spleens of RAG−/− mice in the absence of adoptively transferred L.m.-specific CD45.1+ T cells. In contrast, viable bacteria were undetectable (<10^3 CFUs) after transfer of 100 naive CD45.1+ OT-1 cells, and successful 10-cell and single-cell transfers led to a significant reduction of bacterial load in comparison with mice that had no detectable CD45.1+ progeny. This demonstrates that the lowest numbers of antigen-specific T cells can restrict bacterial growth even in the absence of previous T-cell priming or endogenous T-cell help.

The complete absence of endogenous adaptive immunity in RAG−/− mice could facilitate survival and proliferation after low-dose T-cell transfer due to increased availability of survival factors like interleukin-7 or interleukin-15.35,36 Although clinical adoptive T-cell transfer is often performed under such lymphopenic conditions, we wanted to estimate the influence of homeostatic proliferation in our experimental setting. Therefore, we compared low-dose transfer efficacy rates in RAG−/− and B6 wt mice using the MVA-Ova prime/boost scheme described above (Figure 1A). Ten-cell transfers into B6 wt mice resulted in detectable CD45.1+ T-cell expansions in 85% of all transfers (supplemental Figure 1A) and thus showed identical efficacy rates (67 mice) as transfers into RAG−/− hosts (supplemental Figure 1B). Although the mean absolute numbers of CD45.1+ T cells in spleens of B6 wt recipients served slightly lower (supplemental Figure 1C) than those in RAG−/− mice, this trend was not statistically significant (P = .180). Altogether, antigen-specific naive T cells, transferred in the lowest cell doses, survive and proliferate also in the presence of a physiological T-cell compartment in wt mice.

**Antigen-triggered proliferation and differentiation of CD62Lhi CD8+ memory T cells after low-dose transfer**

Naïve antigen-specific precursor T cells are often very low in frequency and too difficult to detect or enrich from human blood by today’s methods. Therefore, the main focus for clinical adoptive transfers (at least if nonmanipulated primary T cells are used) is currently put on circulating antigen-experienced T cells. Because both CD62Lhi and CD62Llo memory T cells have been described to contribute to protection against reinfections with L.m. in mice,29,37 we examined their survival and differentiation potential after low-dose T-cell transfer (Figure 2). CD45.1+ OT-I memory cells were isolated from L.m.-Ova immune donor mice (CD45.2+) by highly pure FACs sorting of either CD62Llo or CD62Lhi antigen-experienced CD44hi memory T cells (Figure 2A-B). CD62Llo memory T cells showed high survival rates after adoptive transfer, manifesting in successful 10-cell transfers, whereas descendants from CD62Lhi memory T cells could only be detected when recipients had received higher T-cell doses. In addition, expanded populations derived from CD62Llo CD45.1 OT-I T cells exhibited lower
levels of differentiation into long-lasting CD127+ memory T cells (Figure 2C). Furthermore, descendants of CD62Lhi memory T cells were detectable for more than 8 weeks after transfer, indicating long-term persistence (data not shown). In consequence, CD62Lhi memory T cells seem to be the better-suited candidates for prophylactic low-dose transfers.

**Single-cell transfer from polyclonal CD62Lhi CD8+ memory T cells can establish a protective T-cell compartment against high-dose L.m.-Ova infection**

In order to mimic most realistically a potential source of CD62Lhi CD8+ memory T cells for future adoptive T-cell transfers in humans, we isolated polyclonal H2-Kb-SIINFEKL-specific CD62Lhi CD8+ memory T cells using MHC-Streptamers from resting L.m.-Ova–immune CD45.1 mice and tested their protective capacity after adoptive transfer into RAG−/− recipients (Figure 3A). FACS sorting of CD62Lhi H2-Kb-SIINFEKL+ CD45.1+ splenocytes led to the highest purity of enriched cells (Figure 3B; 100% CD62Lhi/CD8+/CD44hi cells gated on living lymphocytes). In order to prevent T-cell activation mediated by MHC-multimer binding to the cognate TCR, the remaining Streptamers were completely removed directly after FACS purification (data not shown).

Similar to naive OT-I T cells, even single memory T cells derived from polyclonal Ova257-264-peptide–specific CD62Lhi CD8+ T-cell populations were able to expand vigorously after in vivo MVA-Ova restimulation and were readily detectable in peripheral blood 3 weeks after transfer (data not shown). Accordingly, a high-dose ($2 \times 10^5$)
**L.m.-Ova challenge** was completely controlled in successfully transferred RAG⁻/⁻ mice manifesting in undetectable bacterial growth 3 days after infection. In spleen, this corresponded to an at least 1000-fold reduction of bacterial burden in comparison with unprotected RAG⁻/⁻ control mice (Figure 3C).

Taken together, the smallest amounts of naive as well as antigen-experienced CD62L⁺ memory CD8⁺ T cells can successfully expand and differentiate after adoptive T-cell transfer and confer protection against otherwise-lethal *L.m.* infections in mice.

**Vigorous proliferation of primary human CMV-specific CD8⁺ T cells after low-dose adoptive T-cell transfer into HSCT patients**

Experience from compassionate-use treatments indicates that HLA-Streptamer–enriched CMV-specific T cells can be detected after transfer into hematopoietic stem cell transplantation (HSCT) recipients, expand, and correlate with control of therapy-refractory CMV reactivation. Here, 2 children with CMV reactivations after HSCT were treated in a compassionate-use setting according to a recently established protocol. Both patients received very low amounts of virus-specific T cells in contrast to previous treatments, allowing the first insights into the course of low-dose T-cell transfers in human immunocompromised patients.

Patient 1 was an 11-month-old boy with SCID syndrome. Born and raised in the middle-eastern region, the severely immunocompromised child suffered from bacille Calmette-Guerin vaccine-induced generalized atypical mycobacteriosis and uncontrolled systemic CMV infection with ocular (retinitis) and cerebral (calcifications) manifestations. A potentially curative haploidentical HSCT with CD34⁺-positive selected stem cells from the father was conducted under CD3-depleting antibody (OKT3) coverage. Because conventional antiviral drug therapy with ganciclovir and foscarnet did not lead to the control of tremendously high (>10⁸ copies per µg DNA) CMV viremia, it was decided to treat the patient by adoptive T-cell transfer from the CMV-seropositive father. Fifteen days after allo-HSCT, CMV-A2-pp65⁺-restricted CD8⁺ T cells were enriched with HLA-Streptamers, and within the same day, the patient received as few as 30 000 antigen-specific T cells (3750 cells per kg body weight) IV. On day 32 after adoptive T-cell transfer, CMV A2-pp65⁺-restricted CD8⁺ T cells became detectable and expanded intensively during the following weeks (Figure 4A). Initial control of CMV blood virus load immediately after transfer was only transient and occurred well before detection of CMV-specific T cells (Figure 4B). Although not examined, this could have been potentially mediated by innate immune cells (eg, natural killer cells). However, temporally rising virus levels decreased drastically for a second time, this time in close correlation with the expanding CMV
A2-pp65–specific T-cell population. During the following weeks, CMV copy levels remained low (Figure 4B). Concomitant side effects (GVHD induction) of the expanding T cells were not observed. CMV A2-pp65-multimer–positive cells stabilized after a peak concentration of nearly 20 cells per mL, which has been previously described as being predictive for antiviral protection. Phenotypic characterization of the expanding CMV A2-pp65-multimer–positive cells showed development from a less differentiated phenotype on day 32 containing CCR7–CD45RA+ central memory phenotype cells (14.5%) to a mature population with a high percentage of so-called Temra cells (CCR7–CD45RA+; supplemental Figure 3C). The establishment of other endogenous CMV-specific T cells did not seem to be hindered by the CMV A2-pp65–specific CD8+ T cells, as CMV A2 IE-1-restricted CD8+ T cells became clearly detectable on day 67 (35 days after the first appearance of the presumably transfer-derived CMV A2-pp65–specific CD8+ T cells).

In order to provide further evidence for the adoptive T-cell transfer as the origin of the detected CMV A2-pp65 CD8 T-cell population, we extracted messenger RNA from FACS-purified CMV A2-pp65-multimer–positive donor T cells and identified in this material a specific TCR Vβ13-CDR3 region sequence. Design of a CDR3 region-specific primer then allowed screening in patient- and donor-derived PBMCs for the identified region and revealed the presence of the donor-specific CDR3 sequence in a posttransfer patient sample (Figure 4C). Resequencing of the products confirmed identity of the products from donor and recipient on the nucleotide level.

Patient 2 was a 14-year-old boy who had initially received cord blood transplantation in second remission after relapsed precursor B-ALL. Because engraftment eventually failed, a second transplantation with haploidentical PBMCs from the father became necessary but was complicated by therapy-refractory CMV reactivation and slow T-cell recovery. In consequence, the patient was treated 5 months after haploidentical HSCT with CMV-specific T cells from the CMV-seropositive father. The boy received only a total of 200,000 A2-pp65–restricted Streptamer-enriched CD8+ T cells (5130 cells per kg body weight), and again, we could observe expansion of CMV A2-pp65-multimer–positive cells after adoptive...
T-cell transfer (Figure 5A). Whereas antigen-specific T-cells proliferated, CMV virus load decreased to very low levels (Figure 5B).

Again, we could detect a donor-specific Vβ13-CDR3 polymerase chain reaction (PCR) product in FACS-sorted CMV A2-pp65–specific CD8+ recipient T cells with a very faint band occurring after 8 weeks of transfer that became clearly detectable 1 week later (9 weeks posttransfer). From this PCR product, donor and recipient identity was again confirmed by sequencing.

Taken together, these 2 clinical cases demonstrate that very small numbers of adoptively transferred CMV-specific Streptamer-enriched CD8+ T cells can cause vigorous expansion and the differentiation of virus-specific T cells in immunocompromised HSCT patients.

### Discussion

Although HSCT has been successfully developed through the last decades and became the standard treatment of various hematopoietic malignancies and primary immune deficiencies, it yet bears a high rate of severe, sometimes lethal complications. Most importantly, substantial risk for acute and chronic GVHD often remains the price to pay with standard transplantation protocols. Principally, depletion of T cells in hematopoietic stem cell transplants can drastically reduce the GVHD risk, but beneficial effects of such protocols were unfortunately found to be counteracted by delayed hematopoietic reconstitution with increased risk for relapse or opportunistic infections. Still, the recent shift in the indication for HSCT toward acute leukemia and/or older age with higher risk for GVHD has renewed the interest in GVHD-minimizing T-cell depletion (TCD) protocols. Indeed, the latest retrospective comparisons of optimized state-of-the-art TCD protocols against conventional GVHD prophylaxis using pharmacologic immunosuppressives suggest that GVHD rates can be significantly reduced without affecting survival rates of related and unrelated donor HSCT. It is tempting to speculate whether successful prevention of viral (and potentially other opportunistic) infections by adoptive T-cell transfer could help to shift the balance in favor of optimized TCD strategies, avoiding the often-limiting side effects (especially in older patients) of antiviral and also immunosuppressive agents (omissible due to the minimized GVHD risk) after transplantation. However, even though omission of pharmacologic immunosuppression in T-cell–depleted HSCT patients should augment the efficacy of transferred antiviral T cells, this clinical situation could, on the other hand, also increase the risk of GVHD induction by contaminating unrestricted CD3+ cells. Because those cells, even under the most stringent purification procedures for virus-specific T cells, cannot be completely eliminated, the applicable numbers of transferred T cells would probably be considerably restricted, particularly if antiviral T cells were applied in a prophylactic manner or isolated from partially HLA-mismatched third-party donors.

In this context, our findings that the lowest doses of pathogen-specific T cells can build up fully differentiated T-cell populations in mice as well as in human HSCT patients indicate that such low-dose transfers could indeed become a successful strategy.

The murine L.m. infection model used here mimics the targeted clinical situation in various ways. First, the complete absence of
endogenous T cells in RAG–/– mice revealed the actual potential of low-dose T-cell transfers in T-cell-deficient lymphopenic hosts. With proper (re-)stimulation either by the replication-deficient MVA or even direct L.m. challenge, very low numbers of transferred L.m.-specific CD8+ T cells proliferated vigorously and differentiated functionally, leaving protective immunity against L.m. challenge. Still, homeostatic proliferation, which has been well described in lymphopenic hosts, could have promoted T-cell survival and expansion after low-dose transfer into RAG–/– mice. However, the immediate antigen-specific stimulation after T-cell transfer makes a main influence of homeostatic proliferation on the extent of subsequent memory T-cell generation unlikely, at least in our experimental setting. Accordingly, the efficacy rates of successful transfers into “full” B6 wt mice were equal to “empty” RAG–/– hosts, although minor influences of the host environment (insignificantly higher amounts of expanded T cells in RAG–/– mice; supplemental Figure 1C) could not be excluded. Even if homeostatic effects favored T-cell expansion in T-cell-deficient hosts, this may well reflect the situation in T-cell-depleted HSCT patients. Interestingly, endogenous CD4+ T cells were not required for the development of protective CD8+ T-cell memory in RAG–/– mice, even though influences on long-term survival of the transferred T cells were not in the focus of our study and remain to be determined. Furthermore, a compensatory contribution of inflammatory stimuli during MVA-Ova stimulation or L.m.-Ova infection in the absence of CD4+ T-cell help cannot be excluded. In any case, in particular interleukin-2–2-producing pathogen-specific memory T cells, which have been originally properly primed in healthy donors, should be well equipped to survive and expand after clinical transfers into immunocompromised hosts.

Importantly, T cells derived from murine polyclonal antigen-specific memory T-cell populations were as protective as naive TCR-transgenic CD8+ T cells, and even single memory cells could develop into fully protective diverse T-cell progeny. This demonstrates that our observations are not limited to TCR-transgenic T cells or a particular TCR. This is crucial for adoptive immunotherapies, because it implicates that human antigen-experienced antiviral T cells, which can control, for example, CMV or Epstein-Barr virus infections in healthy seropositive individuals and which can be reliably selected from blood donors, may also similarly harbor the tremendous expansion potential of their murine T-cell memory counterparts. The low-dose transfers of HLA-Streptamer–enriched CMV-specific CD8+ T cells into 2 patients, which we report here (Figures 4 and 5), indeed support this assumption.

Because the functional reconstitution of a pathogen-specific T-cell compartment will be essential for the protective effect of low-dose transfers in clinical settings, we would suggest to apply those cells as early as possible after HSCT. In prophylactic settings, polyclonal central memory T cells (T CMα) could survive until pathogens start to replicate (supplemental Figure 2), functionally differentiate after antigenic stimulation, and prevent clinical manifestation. Alternatively, very early preemptive usage of low-dose transfers could be envisioned in settings where pathogen replication could be temporally contained by anti-infective medication (eg, CMV reactivation).

We also compared the transfer potential of different T memory subtypes. Intriguingly, L.m.-specific CD62Lhi memory T cells showed a clearly advantageous proliferation and differentiation profile in comparison with CD62Llo memory T cells. In humans, antigen-experienced CD62Lhi (CCR7+) T cells have been originally described as T CMα, distinguishable from naive T cells by the switch from CD45RA to CD45RO expression. They circulate between blood and lymph nodes and show interleukin-15–dependent long-term survival with low turnover but are known to proliferate extensively after antigen re-encounter. They are mainly recruited in case of inefficient antigen clearance by local CD62Lhi effector memory T cells in order to refill the waning effector and effector memory T-cell compartments. How T CMα are generated and sustained during primary and secondary antigen challenge, respectively, is intensively discussed in the field. Recent data from single-cell transfer experiments in mice are in favor of the so-called progressive differentiation model, which postulates an unidirectional developmental pathway from long-lived T CMα to terminally differentiated short-lived effector T cells. In consequence, T CMα-containing antiviral T-cell populations should be the better choice for long-term protective effects as required for prophylactic applications. This would be fully in line with recent studies postulating advantageous (and even stem cell-like) characteristics of relatively undifferentiated human CD62Lhi memory T cells for adoptive T-cell transfer.

Whatever the optimal subset definition for potent CD62Lhi memory T cells might finally be, it will be crucial for prophylactic T-cell products to preserve them during selection, restimulation, or in vitro expansion. Direct transfer of minimally manipulated T cells after gentle ex vivo purification with reversible Streptamers should be very effective for that purpose, as supported by the results from lowest-dose-cell transfers in mice (Figure 3). Because circulating T CMα are found only in small frequencies among human CMV-specific CD8+ T cells, the actual number of transferred T CMα into the HSCT recipients of our study (Figures 4 and 5) must have been extremely low, indicating the potency of direct ex vivo selection of this particular T-cell subset for clinical T-cell transfer strategies. By that, our data imply that in contrast to classical antiviral medication, T-cell therapy does not follow a linear dose-effect relation but can create protective immunity out of the lowest T-cell numbers.

In summary, minimally manipulating (ex vivo) isolation protocols of pathogen-specific T cells, which preserve presumably protective CD62Lhi memory T cells, could be the key to effective but safe prophylactic T-cell transfers in TCD allo-HSCT patients. Prophylactic and preemptive use of an entire MHC-Streptamer–enriched CMV-specific CD8+ T population in allo-HSCT patients is currently being tested in phase 1/2 and 3 trials (Eudra-CT: 2006-006146-34, #NCT01077908 and #NCT01220895). If safe and effective, the recently described ex vivo purification of memory T-cell subsets might become an interesting complementary tool to specifically target the donor-derived CD62Lhi memory subset for “low-dose” adoptive transfer and to extend their prophylactic use in TCD HSCT patients to further (including CD4-restricted) pathogen epitopes and entities.

Acknowledgments

This work was supported by the SFB (Sonderforschungsbereich/ Collaborative Research Centre) TR36 (TP-A10).

Authorship

and B.V. were responsibly involved in patient treatment; D.H.B., H.E., T.T., E.S., and L.G. performed and supervised the clinical cell selection; and M.N., D.H.B., and C.S. wrote the paper.

Conflict-of-interest disclosure: L.G. is a member of and holds shares in Stage Cell Therapeutics Ltd. D.H.B. invented the Streptamer technology and holds shares of Stage Cell Therapeutics Ltd. All other authors declare no competing financial interests.

The current affiliation for G.G. is Howard Hughes Medical Institute and Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY.

Correspondence: Michael Neuenhahn, Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Trotgerstrasse 30, 81675 Munich, Germany; e-mail: michael.neuenhahn@tum.de.

References

34. Wagner JE, Thompson JS, Carter SL, Kernan NA. Unrelated Donor Marrow Transplantation Trial. Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion


Lowest numbers of primary CD8+ T cells can reconstitute protective immunity upon adoptive immunotherapy