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

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Key Points

Lowest numbers of ex vivo selected $CD8^+$ memory T cells can reconstitute pathogen-specific immunity in immuno-compromised hosts.

Abstract

Patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) are threatened by potentially lethal viral manifestations like Cytomegalovirus (CMV) reactivation. Since the success of todays' virostatic treatment is limited by side effects and resistance development, adoptive transfer of virus-specific memory T cells derived from the stem cell donor has been proposed as an alternative therapeutic strategy. In this context, dose minimization of adoptively transferred T cells might be warranted for the avoidance of graft versus host disease (GvHD), in particular in prophylactic settings after T cell-depleting allo-HSCT protocols. To establish a lower limit for successful adoptive T cell therapy, we conducted low dose $CD8^+$ T cell transfers in the well-established murine *Listeria monocytogenes* (*L.m.*) infection model. MHC-Streptamer-enriched antigen-specific $CD62L^{hi}$, but not $CD62L^{lo}$ $CD8^+$ memory T cells proliferated, differentiated and protected against *L.m.* infections after prophylactic application. Even progenies derived from one single $CD62L^{hi}$ *L.m.*-specific $CD8^+$ T cell could be protective against bacterial challenge. In analogy, low dose transfers of Streptamer-enriched human CMV-specific $CD8^+$ T cells into allo-HSCT recipients led to strong pathogen-specific T cell expansion in a compassionate-use setting. In summary, low dose adoptive

T cell transfer could be a promising strategy particularly for prophylactic treatment of infectious complications after allo-HSCT.

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 (EBV), Herpes simplex virus (HSV), Varicella zoster virus (VZV) 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 foscavir 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 non-infectious 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 GMP-conform in vitro expansions[10].

More recently, direct ex vivo isolation (<24^{h}) of virus-specific T cells using conventional[11] or minimally manipulating reversible MHC-multimers[12,13] as well as short time stimulated cytokine secreting T cells[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 GvHD-triggering $CD3^+$ T cells restricts the total number of adoptively transferred T cells[17]. In particular for the envisioned prophylactic strategies or the recently proposed use of partially HLA-matched third party donors[18-20], use of small-sized clinical T cell products might become indispensable to keep the risk of GvHD as low as possible.

Since 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 *Listeria monocytogenes (L.m.)*. After systemic application in mice, *L.m.* uses cellular niches to survive initially in the spleen[21,22]. Although infection is primarily confined by innate defense mechanisms, clearance of *L.m.* depends on the mobilization of adaptive immunity, illustrated by chronic *L.m.* infection in SCID mice[23]. The established (eventually) life-long T cell immunity is mainly mediated by antigen-experienced $CD8^+$ 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 (e.g. $CD62L^{hi}$ 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[24-27].

In addition, we could recently show that single naïve *L.m.*-epitope specific $CD8^+$ T cells can differentiate into diverse effector and memory T cell subsets[28,29]. Here we used a comparable single cell transfer protocol to evaluate the protective capacity of minimal numbers of naïve *L.m.*-epitope specific $CD8^+$ T cells after in vivo challenge and in addition extended these analyses to $CD62L^{hi}$ and $CD62L^{lo}$ $CD8^+$ memory T cells. Lowest numbers of $CD62L^{hi}$ memory T cells developed into diversified progenies conferring protection against *L.m.* challenge, identifying this subpopulation as 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 $CD8^+$ T cells in two compassionate-use allo-HSCT patients.

Methods

Mice and *Listeria monocytogenes* (*L.m.*) infection

$CD45.2^+$ C57BL/6 wildtype (B6 wt) mice were obtained from H. Winkelmann (Borchen, Germany). $CD45.1^+$ congenic C57BL/6 (CD45.1), $CD45.2^+$ RAG1-deficient ($RAG^{-/-}$) mice and $CD45.1^+$ K^b-Ovalbumin peptide ($Ova_{257–264}$ peptide)-specific T cell receptor C57BL/6 transgenic mice (CD45.1-OT-I), were derived from in-house breeding. Experimental conditions of adoptive transfer and L.m. infection experiments are provided in the supplemental Methods, available on the *Blood* Web site)

Isolation of Ovalbumin-specific donor T cells

Naïve CD45.1-OT-I T cells, antigen-experienced $CD45.1^+$ OT-I memory T cells or polyclonal $Ova_{257-264}$-peptide-specific $CD45.1^+$ memory T cells were used for adoptive cell transfer (details in supplemental Methods).

Cell sorting and adoptive transfer of T cells

The adoptive cell transfer of 1-1.000 antigen-specific $CD8^+$ T cells has been previously described[29] and is described in detail in the supplemental Methods.

MVA-Ova immunization and *L.m.*-Ova challenge in recipient mice

Recipient mice were prime-boost immunized by i.v. injection with two subsequent doses (1×10^8 cfu) of a replication-deficient Modified Vaccinia virus type Ankara recombinantly expressing Ovalbumin under control of the viral P7.5 promotor (MVA-Ova)[30]. Expansion and differentiation of T cell progenies were

followed by FACS staining of blood and ex vivo tissue samples as previously described[31] and protective capacity of donor-derived T cell responses was tested in adoptively transferred T-cell-deficient RAG$^{-/-}$ recipient mice by *L.m.*-Ova infection (details in supplemental Methods).

Patients

Two patients were treated with allo-HSCT for SCID-syndrome and 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$^+$ T cells were purified from stem cell donor-derived PBMCs using HLA-Streptamers as previously described[12,13] (details in supplemental Methods).

Tracking of donor-derived CMV HLA-A0201/pp65-specific CD8$^+$ T cells

CDR3 sequencing of ex vivo isolated transferred T cells allowed identification of donor-derived T cells as previously described[13] (details in supplemental Methods).

Approval for the transplantation and the compassionate use treatment was obtained from the Medical Ethical Board (METC) of UMC 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 naïve ovalbumin peptide-specific T cells confers protection against *L.m.*-Ova challenge

In murine *L.m.*-Ova infection, single adoptively transferred ovalbumin peptide-specific $CD8^+$ 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[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 $RAG^{-/-}$ recipient mice[32], in which any functional anti-bacterial T cell response could be unambiguously attributed to the progeny of adoptively transferred T cells. *L.m.*-infected T and B cell-deficient mice are not able to eradicate the pathogen and chronic infection develops[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 ovalbumin-expressing replication-deficient Modified Vaccinia Virus Ankara (MVA-Ova) for prime-boost vaccination prior to challenge with *L.m.*-Ova. $RAG^{-/-}$ mice received a first MVA-Ova dose briefly after adoptive T cell transfer followed by a boost vaccination 14 days later (Fig. 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 15-20% of recipients (data not shown and[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 100-1000-fold higher in recipients with no detectable T cells after single cell transfer and vaccination (Fig. 1B). Taken together, these data show that even lowest amounts of adoptively transferred naïve antigen-specific $CD8^+$ T cells (and in the extreme even one 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 lowest numbers of transferred naïve antigen-specific $CD8^+$ T cells can directly contain bacterial growth in a preemptive setting[33] in $RAG^{-/-}$ mice without previous MVA-Ova vaccination. Mice were infected with a sublethal dose of *L.m.*-Ova immediately after transfer of naïve $CD45.1^+$ OT-I cells and bacterial replication was determined by CFU counts in the spleen nine days later (Fig. 1A). As previously described for *L.m.*-infected SCID mice[23,34], high bacterial numbers (mean 10^5 CFUs, Fig. 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 naïve CD45.1-OT-I cells, and successful 10-cell- and single-cell-transfers led to a significant reduction of bacterial load in comparison to mice that had no detectable $CD45.1^+$ progeny. This demonstrates that lowest numbers of antigen-specific T cells can restrict bacterial growth even in 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 IL-7 or IL-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 (Fig. 1A). 10-cell transfers into B6 wt mice resulted in detectable $CD45.1^{+}$ T cell expansions in 85% of all transfers (A) and thus showed identical efficacy rates (6/7 mice) as transfers into $RAG^{-/-}$ hosts (Fig. S1B). While the mean absolute numbers of $CD45.1^{+}$ T cells in spleens of B6 wt recipients seemed slightly lower (Fig. S1C) than that in $RAG^{-/-}$ mice, this trend was not statistically significant ($p = 0.180$). Altogether, antigen-specific naïve T cells, transferred in lowest cell doses, survive and proliferate also in the presence of a physiological T cell compartment in wild type mice.

Antigen-triggered proliferation and differentiation of $CD62L^{hi}$ $CD8^{+}$ 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 non-manipulated primary T cells are used – is currently put on circulating antigen-experienced T cells. Since both $CD62L^{hi}$ and $CD62L^{lo}$ 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 (Fig. 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 $CD62L^{hi}$ or $CD62L^{lo}$ antigen-experienced $CD44^{hi}$ memory T cells (Fig. 2A and B). $CD62L^{hi}$ memory T cells showed high survival rates after adoptive transfer, manifesting in successful 10-cell transfers, whereas descendants from $CD62L^{lo}$ memory T cells could only be

detected when recipients had received higher T cell doses. In addition, expanded populations derived from CD62L^{lo} CD45.1-OT-I T cells exhibited lower levels of differentiation into long-lasting CD127^{+} memory T cells (Fig. 2C). Furthermore, descendants of CD62L^{hi} memory T cells were detectable for more than eight weeks after transfer indicating long term persistence (data not shown). In consequence, CD62L^{hi} memory T cells seem to be the better-suited candidates for prophylactic low dose transfers.

Single cell transfer from polyclonal CD62L^{hi} 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 CD62L^{hi} CD8^{+} memory T cells for future adoptive T cell transfers in humans, we isolated polyclonal H2-K^{b}-SIINFEKL-specific CD62L^{hi} 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 (Fig. 3A). FACS-sorting of CD62L^{hi} H2-K^{b}-SIINFEKL $^{+}$ CD45.1^{+} splenocytes led to highest purity of enriched cells (Fig. 3B; 100% CD62L^{hi}/CD8^{+}/CD44^{hi} cells gated on living lymphocytes). In order to prevent T cell activation mediated by MHC-multimer binding to the cognate TCR, remaining Streptamers were completely removed directly after FACS-purification (data not shown)[12].

Similar to naïve OT-I T cells, even single memory T cells derived from polyclonal Ova$_{257-264}$-peptide-specific CD62L^{hi} 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 x 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 to unprotected $RAG^{-/-}$ control mice (Fig. 3C).

Taken together, smallest amounts of naïve as well as antigen-experienced $CD62L^{hi}$ 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 indicate that HLA-Streptamer-enriched CMV-specific T cells can be detected after transfer into HSCT recipients, expand and correlate with control of therapy-refractory CMV reactivation. Here two children with CMV reactivations after HSCT were treated in a compassionate-use setting according to a recently established protocol[13]. Both patients received very low amounts of virus-specific T cells in contrast to previous treatments, allowing first insights into the course of low dose T cell transfers in human immunocompromised patients.

Patient #1 was an 11 months old boy with SCID syndrome. Born and raised in the middle-eastern region, the severely immunocompromised child suffered from BCG vaccine-induced generalized atypical mycobacteriosis and uncontrolled systemic CMV infection with ocular (retinitis) and cerebral (calcifications) manifestations. A potentially curative haplo-identical HSC

transplantation with CD34-positive selected stem cells from the father was conducted under CD3-depleting antibody (OKT3) coverage. Since conventional antiviral drug therapy with ganciclovir and foscavir did not lead to the control of tremendously high (>10^8 copies/µg DNA) CMV viremia, it was decided to treat the patient by adoptive T cell transfer from the CMV-seropositive father. 15 days after allo-HSCT, CMV-specific 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 / kg body weight) i.v.. On day 32 after adoptive T cell transfer of CMV A2-pp65-restricted $CD8^+$ T cells became detectable and expanded intensively during the following weeks (Fig. 4A). Initial control of CMV blood virus load immediately after transfer was only transient and occurred well before detection of CMV-specific T cells (Fig. 4B). Although not examined, this could have been potentially mediated by innate immune cells (e.g. NK cells[38,39]). 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 copies remained on low levels (Fig. 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/µl on a level of around 10 cells/µl, which has been previously described as being predictive for antiviral protection[40]. 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 ($CCR7^-CD45RA^+$; Suppl. Fig. 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 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 mRNA from FACS-purified CMV A2 pp65-multimer-positive donor T cells and identified in this material a specific TCR Vß13-CDR3 region sequence. Design of a 3' 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 post-transfer patient sample (Fig. 4C). Re-sequencing of the products confirmed identity of the products from donor and recipient on the nucleotide level.

Patient #2 was a 14 years old boy who had initially received cord blood transplantation in second remission after relapsed precursor B-ALL. Since 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 five 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 / kg body weight) and again, we could observe expansion of CMV A2 pp65-multimer positive cells after adoptive T cell transfer (Fig. 5A). While antigen-specific T cells proliferated, CMV virus load decreased to very low levels (Fig. 5B).

Again, we could detect a donor-specific Vβ13-CDR3 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 one week

later (9 weeks post transfer). From this PCR product donor and recipient identity was again confirmed by sequencing.

Taken together, these two 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 for 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 remains often the price to pay with standard transplantation protocols. Principally, depletion of T cells in HSC transplants can drastically reduce GvHD risk[41], but beneficial effects of such protocols had been unfortunately found to be counteracted by delayed hematopoietic reconstitution with increased risk for relapse or opportunistic infections[42]. Still, the recent shift in indication for HSCT towards acute leukemia and/or older age with higher risk for GvHD has renewed the interest in GvHD-minimizing T cell depletion (TCD) protocols[43]. And indeed, 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[44,45]. 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. And since 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 be probably considerably restricted; in particular, if antiviral T cells were applied in a prophylactic manner or isolated from partially HLA-mismatched “third party” donors[18-20,46].

In this context, our findings that lowest doses of pathogen-specific T cells can build up fully differentiated T cell populations in mice as well as human HSCT patients indicate that such low dose transfers could become indeed 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[47], 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 – at least in our experimental setting – unlikely. Accordingly, the efficacy rates of successful transfers into “full” B6 wild type mice were equal to “empty” $RAG^{-/-}$ hosts, although minor influences of the host environment (insignificantly higher amounts of expanded T cells in $RAG^{-/-}$ mice, Fig. S1C) could not be excluded. But 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 IL-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[28,48].

Importantly, T cells derived from murine polyclonal antigen-specific memory T cell populations were as protective as naïve TCR-transgenic $CD8^+$ T cells, and even single memory cells could develop into fully protective diverse T cell progenies. This demonstrates that our observations are not limited to TCR-transgenic T cells or a particular TCR. This is crucial for adoptive immunotherapies, as it implicates that also human antigen-experienced antiviral T cells, which can control for example CMV or EBV infections in healthy seropositive individuals and which can be reliably selected from blood donors, may 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 two patients, which we report here (Fig. 4 and 5) indeed support this assumption.

Since the functional reconstitution of a pathogen-specific T cell compartment will be essential for the protectivity of low dose transfers in clinical settings, we would suggest to apply those cells as early as possible after HSCT. In prophylactic settings, polyspecific T_{CM} could survive until pathogens start to replicate (Fig. S2), functionally differentiate after antigenic stimulation and prevent finally 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 (e.g. CMV reactivation).

We also compared the transfer potential of different T memory subtypes. Intriguingly, *L.m.*-specific CD62L^{hi} memory T cells showed a clearly advantageous proliferation and differentiation profile in comparison to CD62L^{lo} memory T cells. In humans, antigen-experienced CD62L^{hi} (CCR7^{+}) T cells have been originally described as central memory T cells (T_{CM}), distinguishable from naïve T cells by switch from CD45RA to CD45RO expression[49]. They circulate between blood and lymph nodes, show IL-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 CD62L^{lo} effector memory T cells (T_{EM}) in order to refill the waning effector (T_E) and T_{EM} compartments[50]. How T_{CM} are generated and sustained during primary and secondary antigen challenge, respectively, is intensively discussed in the field [51]. 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_{CM} to terminally-differentiated short-lived T_E cells[28]. In consequence, T_{CM}-containing antiviral T cell populations should be the better choice for long-term protectiveness 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 CD62L^{hi} memory T cells for adoptive T cell transfer[26,27,52]. Whatever the optimal subset definition for potent CD62L^{hi} T memory cells might finally be, it will be crucial for prophylactic T cell products to preserve them during selection, re-stimulation or in vitro expansion[52]. 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 cell dose transfers in mice (Fig. 3). Since circulating T_{CM} are found only in small frequencies among human CMV-specific $CD8^+$ T cells[53], the actual number of transferred T_{CM} into the HSCT recipients of our study (Fig. 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 implicate 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 lowest cell numbers.

In summary, minimally manipulating (ex vivo) isolation protocols of pathogen-specific T cells, which preserve presumably protective $CD62L^{hi}$ 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 tested in phase I/II and III trials (Eudra-CT: 2006-006146-34, NCT01077908 and NCT01220895). If safe and effective, the recently described ex vivo purification of memory T cell subsets[54], might become an interesting complementary tool to specifically target the donor-derived $CD62L^{hi}$ 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.

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Authorship Contributions

M.N., C.S., P.G., G.D., J.A., M.O., F.A., V.B. and M.S. performed experiments; C.S., M.N., M.O. and J.A. analyzed the data; G.G. provided MVA; M.N., C.S. and D.H.B. conceived the study and planned the murine experiments; G.U.G., F.R.S., A.B 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. M.N., D.H.B. and C.S. wrote the paper.

Disclosure of Conflicts of Interest

L.G. is a member of STAGE Cell Therapeutics Ltd. and holds shares of 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.

References

1. Boeckh M, Geballe AP. Cytomegalovirus: pathogen, paradigm, and puzzle. *J. Clin. Invest.* 2011;121(5):1673–1680.
2. Bochud P-Y, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N. Engl. J. Med.* 2008;359(17):1766–1777.
3. Blyth E, Clancy L, Simms R, et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. *Blood*. 2013;121(18):3745–3758.
4. Riddell SR, Watanabe KS, Goodrich JM, et al. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science*. 1992;257(5067):238–241.
5. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N. Engl. J. Med.* 1995;333(16):1038–1044.
6. Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet*. 1995;345(8941):9–13.
7. Heslop HE, Ng CY, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. *Nat. Med.* 1996;2(5):551–555.
8. June CH. Adoptive T cell therapy for cancer in the clinic. *J. Clin. Invest.* 2007;117(6):1466–1476.
9. Jolink H, Meijssen IC, Hagedoorn RS, et al. Characterization of the T-cell mediated immune response against the Aspergillus fumigatus proteins Crf1 and Catalase1 in healthy individuals. *J. Infect. Dis.* 2013.
10. Abou-El-Enein M, Römhild A, Kaiser D, et al. Good Manufacturing Practices (GMP) manufacturing of advanced therapy medicinal products: a novel tailored model for optimizing performance and estimating costs. *Cytotherapy*. 2013;15(3):362–383.
11. Cobbold M, Khan N, Pourgheysari B, et al. Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. *J. Exp. Med.* 2005;202(3):379–386.
12. Knabel M, Franz TJ, Schiemann M, et al. Reversible MHC multimer staining for functional isolation of T-cell populations and effective adoptive transfer. *Nat. Med.* 2002;8(6):631–637.
13. Schmitt A, Tonn T, Busch DH, et al. Adoptive transfer and selective reconstitution of streptamer-selected cytomegalovirus-specific CD8+ T cells leads to virus clearance in patients after allogeneic peripheral blood stem cell transplantation. *Transfusion*. 2011;51(3):591–599.
14. Peggs KS, Thomson K, Samuel E, et al. Directly selected cytomegalovirus-reactive donor T cells confer rapid and safe systemic reconstitution of virus-specific immunity following stem cell transplantation. *Clin. Infect. Dis.* 2011;52(1):49–57.
15. Moosmann A, Bigalke I, Tischer J, et al. Effective and long-term control of

EBV PTLD after transfer of peptide-selected T cells. *Blood*. 2010;115(14):2960–2970.
16. Feuchtinger T, Opherk K, Bethge WA, et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. *Blood*. 2010;116(20):4360–4367.
17. Stemberger C, Neuenhahn M, Gebhardt FE, et al. Stem cell-like plasticity of naïve and distinct memory CD8+ T cell subsets. *Semin. Immunol.* 2009;21(2):62–68.
18. Barker JN, Doubrovina E, Sauter C, et al. Successful treatment of EBV-associated posttransplantation lymphoma after cord blood transplantation using third-party EBV-specific cytotoxic T lymphocytes. *Blood*. 2010;116(23):5045–5049.
19. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood*. 2007;110(4):1123–1131.
20. Uhlin M, Gertow J, Uzunel M, et al. Rapid salvage treatment with virus-specific T cells for therapy-resistant disease. *Clin. Infect. Dis.* 2012;55(11):1064–1073.
21. Neuenhahn M, Kerksiek KM, Nauerth M, et al. CD8alpha+ dendritic cells are required for efficient entry of Listeria monocytogenes into the spleen. *Immunity*. 2006;25(4):619–630.
22. Verschoor A, Neuenhahn M, Navarini AA, et al. A platelet-mediated system for shuttling blood-borne bacteria to CD8α+ dendritic cells depends on glycoprotein GPIb and complement C3. *Nat. Immunol.* 2011;12(12):1194–1201.
23. Bhardwaj V, Kanagawa O, Swanson PE, Unanue ER. Chronic Listeria infection in SCID mice: requirements for the carrier state and the dual role of T cells in transferring protection or suppression. *J. Immunol.* 1998;160(1):376–384.
24. Neuenhahn M, Busch DH. The quest for CD8+ memory stem cells. *Immunity*. 2009;31(5):702–704.
25. Wang X, Berger C, Wong CW, et al. Engraftment of human central memory-derived effector CD8+ T cells in immunodeficient mice. *Blood*. 2011;117(6):1888–1898.
26. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell-like properties. *Nat. Med.* 2011;17(10):1290–1297.
27. Berger C, Jensen MC, Lansdorp PM, et al. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J. Clin. Invest.* 2008;118(1):294–305.
28. Buchholz VR, Flossdorf M, Hensel I, et al. Disparate individual fates compose robust CD8+ T cell immunity. *Science*. 2013;340(6132):630–635.
29. Stemberger C, Huster KM, Koffler M, et al. A single naive CD8+ T cell precursor can develop into diverse effector and memory subsets. *Immunity*. 2007;27(6):985–997.
30. Kastenmuller W, Gasteiger G, Gronau JH, et al. Cross-competition of CD8+ T cells shapes the immunodominance hierarchy during boost

vaccination. *J. Exp. Med.* 2007;204(9):2187–2198.
31. Schiemann M, Busch V, Linkemann K, Huster KM, Busch DH. Differences in maintenance of CD8+ and CD4+ bacteria-specific effector-memory T cell populations. *Eur. J. Immunol.* 2003;33(10):2875–2885.
32. Mombaerts P, Iacomini J, Johnson RS, et al. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell*. 1992;68(5):869–877.
33. Pahl-Seibert M-F, Juelch M, Podlech J, et al. Highly protective in vivo function of cytomegalovirus IE1 epitope-specific memory CD8 T cells purified by T-cell receptor-based cell sorting. *J. Virol.* 2005;79(9):5400–5413.
34. Pope C, Kim SK, Marzo A, et al. Organ-specific regulation of the CD8 T cell response to Listeria monocytogenes infection. *J. Immunol.* 2001;166(5):3402–3409.
35. Schluns KS, Kieper WC, Jameson SC, Lefrançois L. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. *Nat. Immunol.* 2000;1(5):426–432.
36. Sandau MM, Winstead CJ, Jameson SC. IL-15 is required for sustained lymphopenia-driven proliferation and accumulation of CD8 T cells. *J. Immunol.* 2007;179(1):120–125.
37. Huster KM, Stemberger C, Busch DH. Protective immunity towards intracellular pathogens. *Curr. Opin. Immunol.* 2006;18(4):458–464.
38. Muñoz-Cobo B, Solano C, Benet I, et al. Functional profile of cytomegalovirus (CMV)-specific CD8+ T cells and kinetics of NKG2C+ NK cells associated with the resolution of CMV DNAemia in allogeneic stem cell transplant recipients. *J. Med. Virol.* 2012;84(2):259–267.
39. Barron MA, Gao D, Springer KL, et al. Relationship of reconstituted adaptive and innate cytomegalovirus (CMV)-specific immune responses with CMV viremia in hematopoietic stem cell transplant recipients. *Clin. Infect. Dis.* 2009;49(12):1777–1783.
40. Gratama JW, van Esser JW, Lamers CH, et al. Tetramer-based quantification of cytomegalovirus (CMV)-specific CD8+ T lymphocytes in T-cell-depleted stem cell grafts and after transplantation may identify patients at risk for progressive CMV infection. *Blood*. 2001;98(5):1358–1364.
41. Devine SM, Carter S, Soiffer RJ, et al. Low risk of chronic graft-versus-host disease and relapse associated with T cell-depleted peripheral blood stem cell transplantation for acute myelogenous leukemia in first remission: results of the blood and marrow transplant clinical trials network protocol 0303. *Biol. Blood Marrow Transplant.* 2011;17(9):1343–1351.
42. 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 Trial): a multi-centre, randomised phase II-III trial. *Lancet*. 2005;366(9487):733–741.
43. Giralt S. Graft-versus-host disease: have we solved the problem? *J. Clin. Oncol.* 2012;30(26):3160–3161.
44. Pasquini MC, Devine S, Mendizabal A, et al. Comparative outcomes of

donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. *J. Clin. Oncol.* 2012;30(26):3194–3201.

45. Bayraktar UD, de Lima M, Saliba RM, et al. Ex Vivo T Cell Depleted versus Unmodified Allografts in Patients with Acute Myeloid Leukemia in First Complete Remission. *Biol. Blood Marrow Transplant.* 2013.
46. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood*. 2013;121(26):5113–5123.
47. Takada K, Jameson SC. Naive T cell homeostasis: from awareness of space to a sense of place. *Nat. Rev. Immunol.* 2009;9(12):823–832.
48. Feau S, Arens R, Togher S, Schoenberger SP. Autocrine IL-2 is required for secondary population expansion of CD8(+) memory T cells. *Nat. Immunol.* 2011;12(9):908–913.
49. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401(6754):708–712.
50. Mueller SN, Gebhardt T, Carbone FR, Heath WR. Memory T cell subsets, migration patterns, and tissue residence. *Annu. Rev. Immunol.* 2013;31:137–161.
51. Williams MA, Bevan MJ. Effector and memory CTL differentiation. *Annu. Rev. Immunol.* 2007;25:171–192.
52. Schmueck M, Fischer AM, Hammoud B, et al. Preferential expansion of human virus-specific multifunctional central memory T cells by partial targeting of the IL-2 receptor signaling pathway: the key role of CD4+ T cells. *J. Immunol.* 2012;188(10):5189–5198.
53. Appay V, van Lier RAW, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A*. 2008;73(11):975–983.
54. Stemberger C, Dreher S, Tschulik C, et al. Novel serial positive enrichment technology enables clinical multiparameter cell sorting. *PLoS ONE*. 2012;7(4):e35798.

Figure Legends

Fig. 1: Adoptive transfer of a single naive antigen-specific CD8$^+$ T cell can reconstitute protective immunity towards high dose *Listeria monocytogenes* (*L.m.*) infection.

(A) Schematic outline of the experimental procedure. RAG$^{-/-}$ recipient mice received a single naïve (CD44^{lo}) CD45.1-OT-I CD8$^+$ T cell by i.p. application. On days 0 and 14 post T cell transfer, prime/boost i.v. vaccination was performed with 1 x 10^8 MVA-Ova. On day 21 after T cell transfer, mice were challenged intravenously with an otherwise lethal dose of *L.m.-Ova*. Three days later, viable bacteria in tissue homogenates were determined by counting CFUs on BHI plates. Results are depicted in (B). Alternatively, RAG$^{-/-}$ recipient mice received naïve CD45.1-OT-I TCR-transgenic CD8$^+$ T cell as before and on the same day recipient mice were challenged intravenously with *L.m.*-Ova (infection dose 7500 bacteria). Nine days later, viable bacteria (as shown in (C)) were determined in spleen and liver. Mice receiving 100 CD45.1 OT-I cells served as positive control, while mice with undetectable CD45.1 OT-I T cells after single cell transfer (no cells) served as negative control (n.d. = not detectable). Horizontal bars indicate means, P values calculated by 1way ANOVA.

Fig. 2: Expansion of CD62L^{hi} antigen-experienced memory T cells after low dose T cell transfer.

(A) B6 wt (CD45.2) recipient mice received 10 naïve CD44^{lo} CD45.1$^+$ OT-I T cells, and were subsequently infected with 5 x 10^3 *L. m.*-Ova. Eight months

later, CD45.1^{+} OT-I T cells were identified from living lymphocytes as CD44^{hi} CD62L^{hi} and CD62L^{lo} memory T cell subsets (B, before cell sorting). Subset cells were FACS-purified (B, after cell sorting) and transferred into *L.m.*-Ova-infected (5 x 10^{3}) B6 wt (CD45.2) recipient mice, respectively. Expansion and differentiation (C) of the transferred memory T cell subsets were analyzed twelve days later. The frequencies of re-expanded CD45.1 OT-I memory subset T cells in the spleen after transfer of the indicated cell numbers are demonstrated and representative differentiation patterns of expanded CD45.1^{+} T cells (CD127 and CD62L staining) are shown (n.d. = not detectable).

Fig. 3: Successful single cell transfer of CD62L^{hi} antigen-experienced CD8^{+} memory T cells.

(A) Adoptive transfer protocol from H2-K^{b}/SIINFEKL-Streptamer-enriched polyclonal (not TCR transgenic) memory T cells. (B) MHC-Streptamer-positive CD62L^{hi} CD8^{+} memory T cells were identified in spleens of *L.m.*-Ova-immune CD45.1 wild type mice (B, before cell sorting) and FACS-purified (purities in B, after cell sorting). Streptamer reagents were removed after addition of D-biotin and cells were immediately transferred into RAG$^{-/-}$ mice. Recipient mice were MVA-vaccinated and *L.m.*-Ova-challenged in analogy to Fig. 1. (C) Bacterial counts in spleen and liver of mice with the indicated transferred T cell numbers are shown (n.d. = not detectable). Negative control mice had undetectable CD45.1-OT-I T cells after single cell transfer (no cells).

Fig. 4: Expansion of CMV-specific CD8$^+$ T cells after low dose T cell transfer in a SCID patient.

A ten months old boy with SCID syndrome and generalized CMV disease (patient #1) had been reconstituted with PBSCs from the father. Fourteen days after allo-HSCT, 30.000 donor-derived CMV HLA-A0201/pp65 peptide-specific CD8$^+$ T cells (3750 per kg body weight) were infused. Patient-derived PBMCs were analyzed at different time points before and after adoptive transfer. (A) Visualization of CMV HLA-A0201/pp65 peptide-specific T cells using MHC-multimers. The frequencies among CD3$^+$ T cells are indicated. Additionally, the kinetics of endogenously selected CMV HLA-A0201/IE-1 peptide-specific CD8$^+$ T cells of respective time points are illustrated (n.p. = not performed). (B) Comparison of CMV-specific T cell kinetics and CMV detection. The absolute numbers of CMV HLA-A0201/pp65 peptide-specific T cells (circles) are indicated. CMV load was measured in the peripheral blood via quantitative PCR (filled gray). (C) Tracking of donor-derived CMV HLA-A0201/pp65-specific CD8$^+$ T cells. Amplified donor and patient PCR products of an identified CDR3 region are shown (left). In control PBMCs, no product amplification was detectable. Detected PCR products (ca. 193 bp) were subsequently sequenced. The isolated sequences of the CDR3 region from patient and donor are shown in detail (right; blue: V segment; green: D segment; red: J segment).

Fig. 5: Proliferation of CMV-specific CD8$^+$ T cells in a patient with haploidentical HSCT after B-ALL.

A fourteen-year-old boy with B-ALL (patient #2) and therapy-refractory CMV

reactivation after haploidentical allo-HSCT was treated with Streptamer-purified CMV-specific $CD8^+$ T cells. He received 5130 cells /kg (in total 200.000 cells) stem cell donor-derived CMV HLA-A0201/pp65 peptide-specific T cells five months after allo-HSCT. Patient-derived PBMCs were analyzed at different time points before and after adoptive transfer. (A) CMV HLA-A0201/pp65 peptide-specific T cells were visualized with MHC-multimers and selected time points are demonstrated. The frequencies among $CD3^+$ T cells are indicated. (B) Comparison of CMV-specific T cell kinetics and CMV detection. The frequency of CMV HLA-A0201/pp65 peptide-specific cells among $CD3^+$ T cells is indicated (circles). CMV load was measured in the peripheral blood via quantitative PCR (filled gray). (C) Tracking of donor-derived CMV HLA-A0201/pp65-specific $CD8^+$ T cells via CDR3 sequencing. Amplified donor and patient PCR products (8 and 9 weeks) of an identified CDR3 region are shown (top). Detected PCR products were subsequently sequenced. The isolated sequences of the CDR3 region from patient and donor are shown in detail (bottom; blue: V segment; green: D segment; red: J segment).

Fig. 1

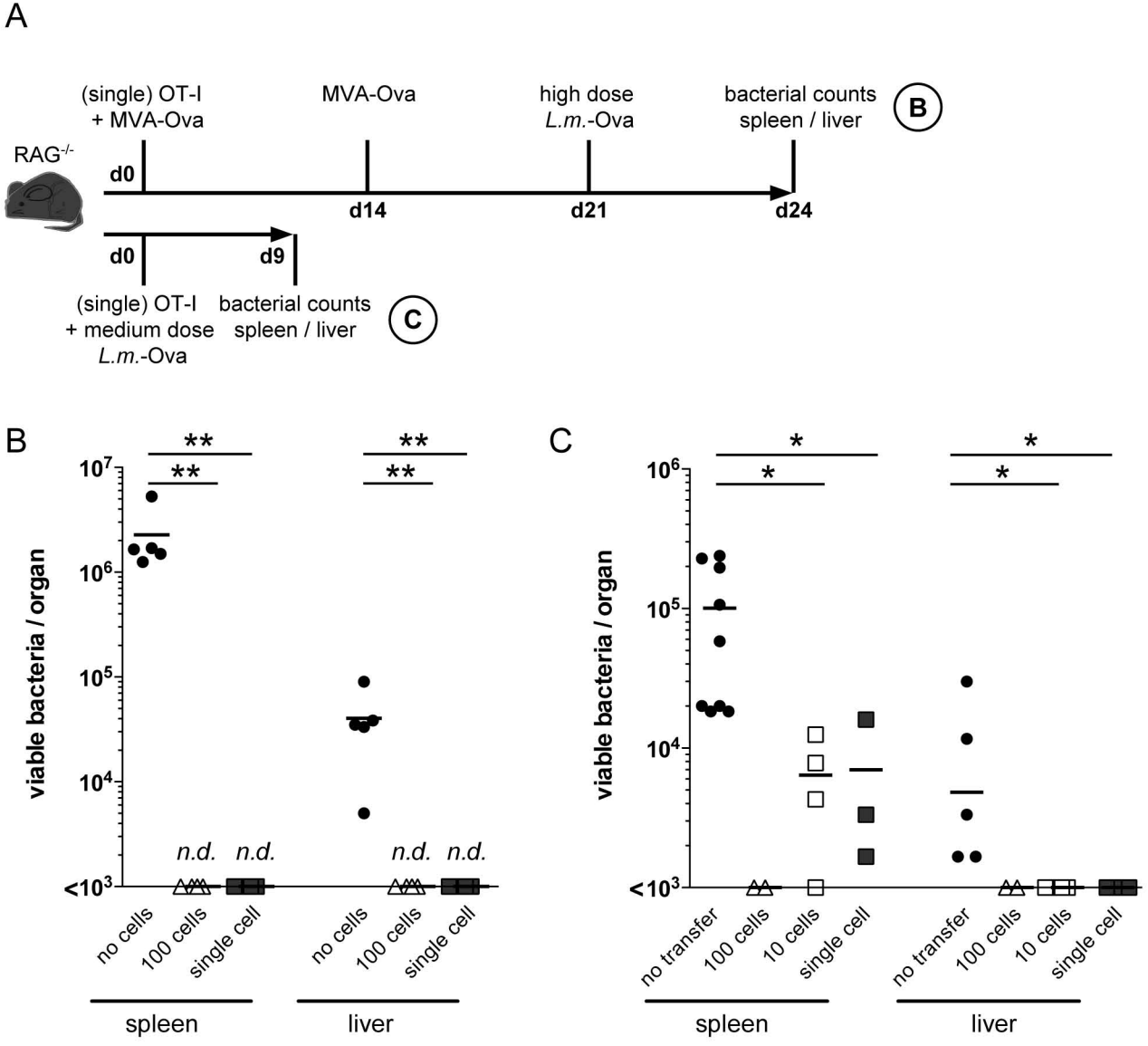

A
(single) OT-I
+ MVA-Ova
MVA-Ova
high dose
L.m.-Ova
bacterial counts
spleen / liver
B
RAG-/-
d0
d14
d21
d24
d0
d9
(single) OT-I
+ medium dose
L.m.-Ova
bacterial counts
spleen / liver
C
B
viable bacteria / organ
10^7
10^6
10^5
10^4
<10^3
n.d.
n.d.
n.d.
n.d.
no cells
100 cells
single cell
no cells
100 cells
single cell
spleen
liver
C
viable bacteria / organ
10^6
10^5
10^4
<10^3
no transfer
100 cells
10 cells
single cell
no transfer
100 cells
10 cells
single cell
spleen
liver

Fig. 2

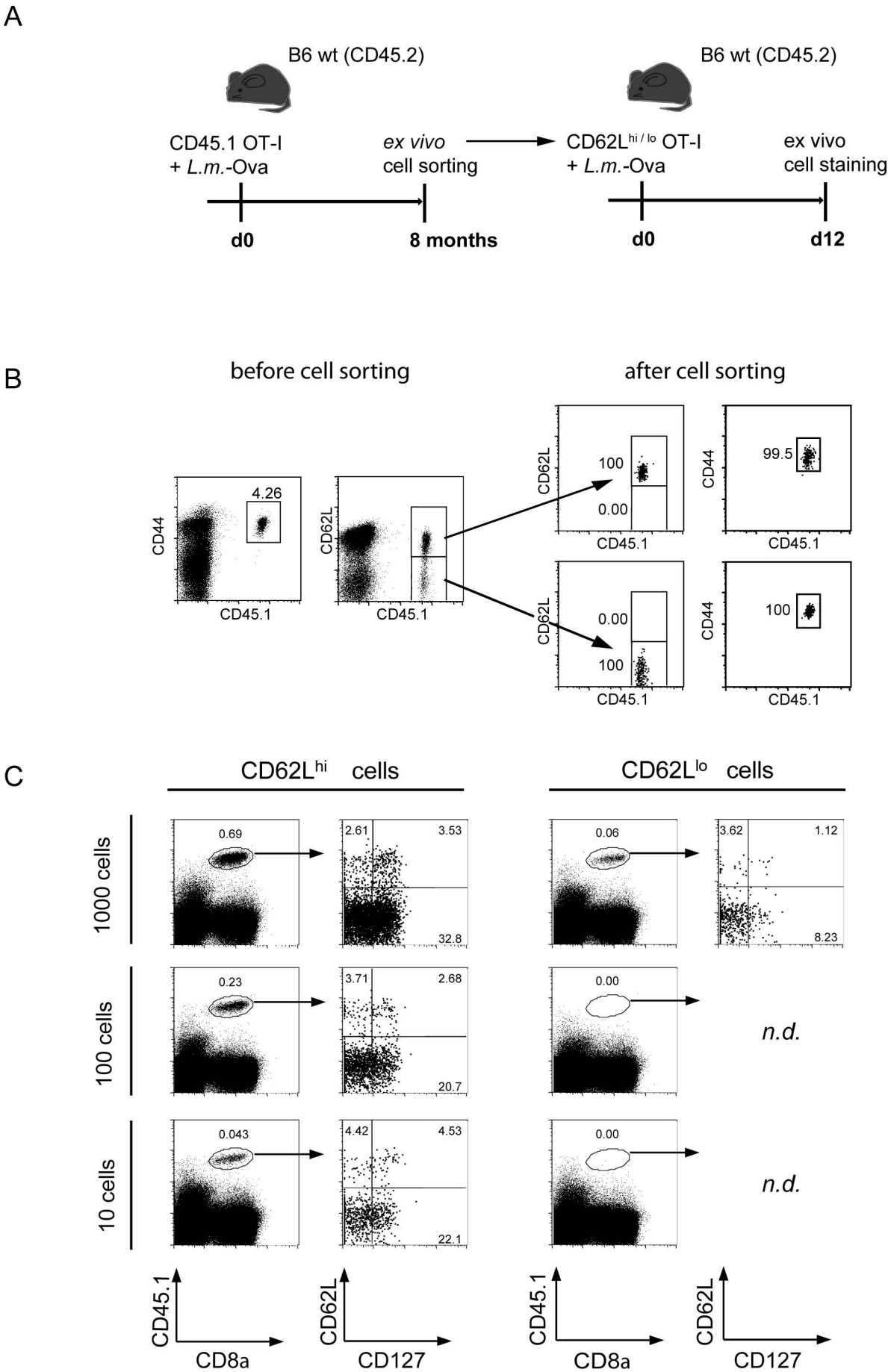

A
B6 wt (CD45.2)
B6 wt (CD45.2)
CD45.1 OT-I
+ L.m.-Ova
ex vivo
cell sorting
CD62Lhi / lo OT-I
+ L.m.-Ova
ex vivo
cell staining
d0
8 months
d0
d12
B
before cell sorting
after cell sorting
CD44
CD45.1
4.26
CD62L
CD45.1
CD62L
CD45.1
100
0.00
CD44
CD45.1
99.5
CD62L
CD45.1
0.00
100
CD44
CD45.1
100
C
CD62Lhi cells
CD62Llo cells
1000 cells
100 cells
10 cells
0.69
2.61
3.53
32.8
0.06
3.62
1.12
8.23
0.23
3.71
2.68
20.7
0.00
n.d.
0.043
4.42
4.53
22.1
0.00
n.d.
CD45.1
CD8a
CD62L
CD127
CD45.1
CD8a
CD62L
CD127

Fig. 3

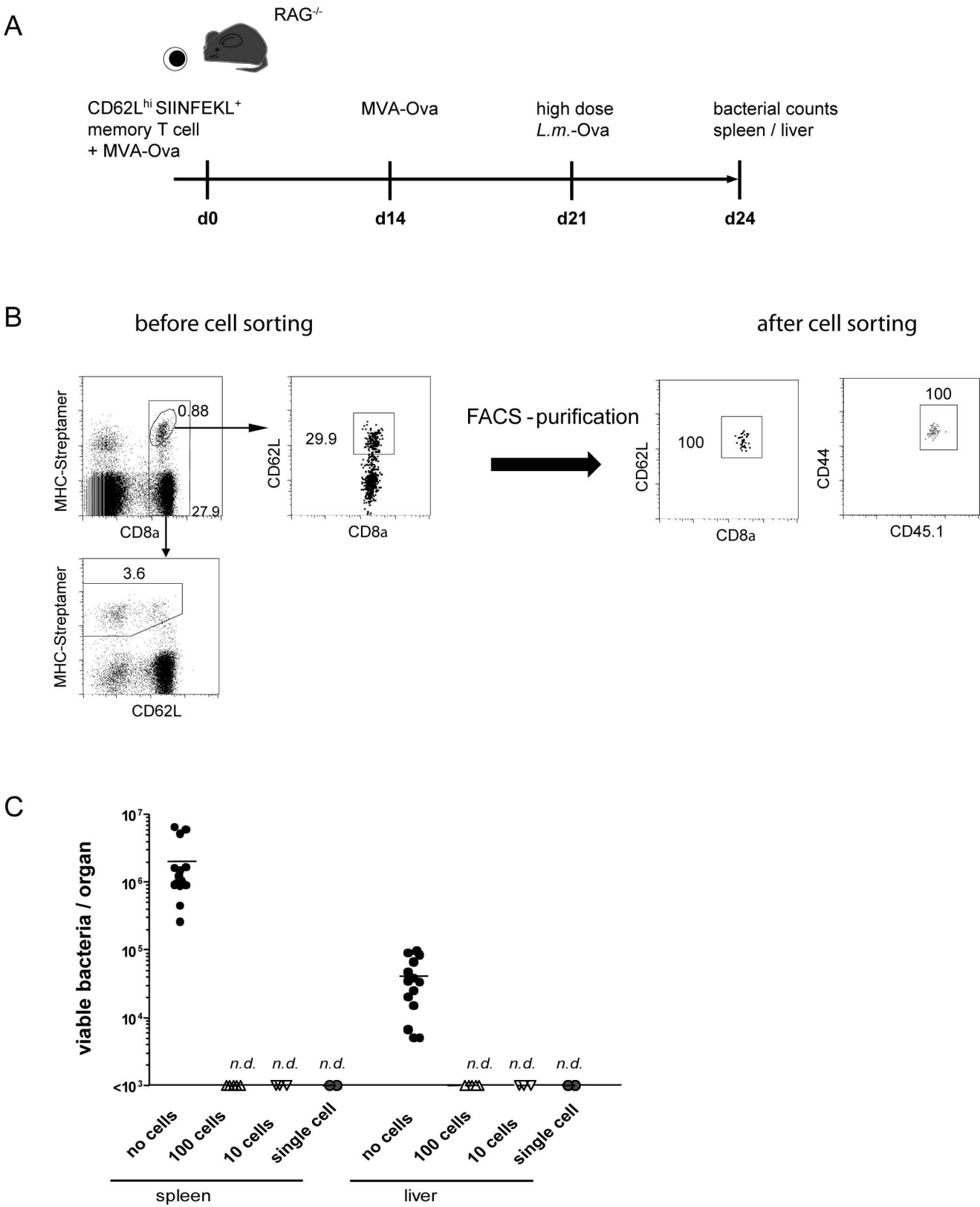
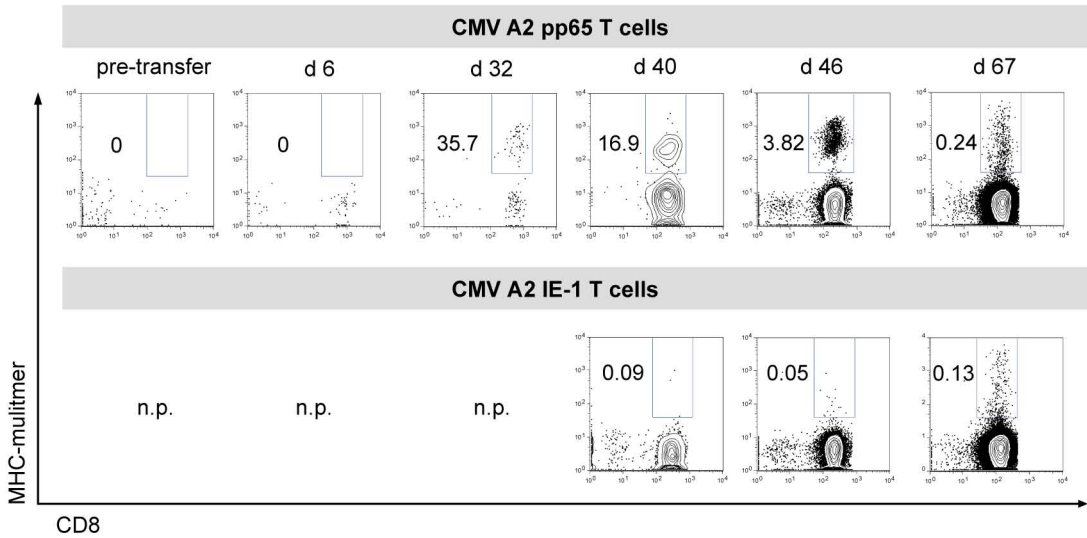

A
RAG-/-
CD62Lhi SIINFEKL+
memory T cell
+ MVA-Ova
MVA-Ova
high dose
L.m.-Ova
bacterial counts
spleen / liver
d0
d14
d21
d24
B
before cell sorting
after cell sorting
MHC-Streptamer
0.88
27.9
CD8a
CD62L
29.9
CD8a
FACS - purification
CD62L
100
CD8a
CD44
100
CD45.1
MHC-Streptamer
3.6
CD62L
C
viable bacteria / organ
10^7
10^6
10^5
10^4
<10^3
n.d.
n.d.
n.d.
n.d.
n.d.
n.d.
no cells
100 cells
10 cells
single cell
no cells
100 cells
10 cells
single cell
spleen
liver

Fig. 4

A



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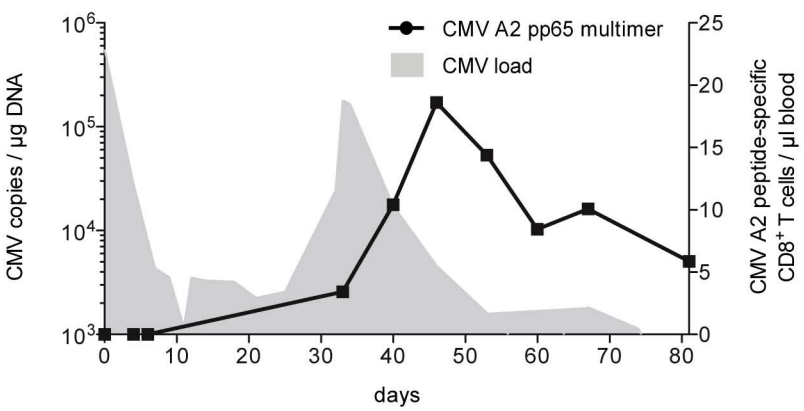

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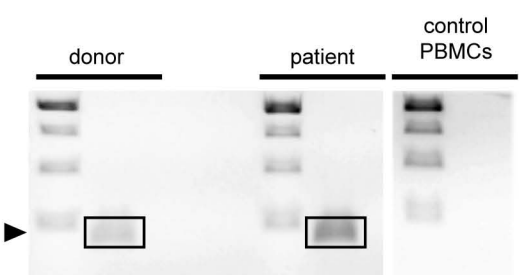


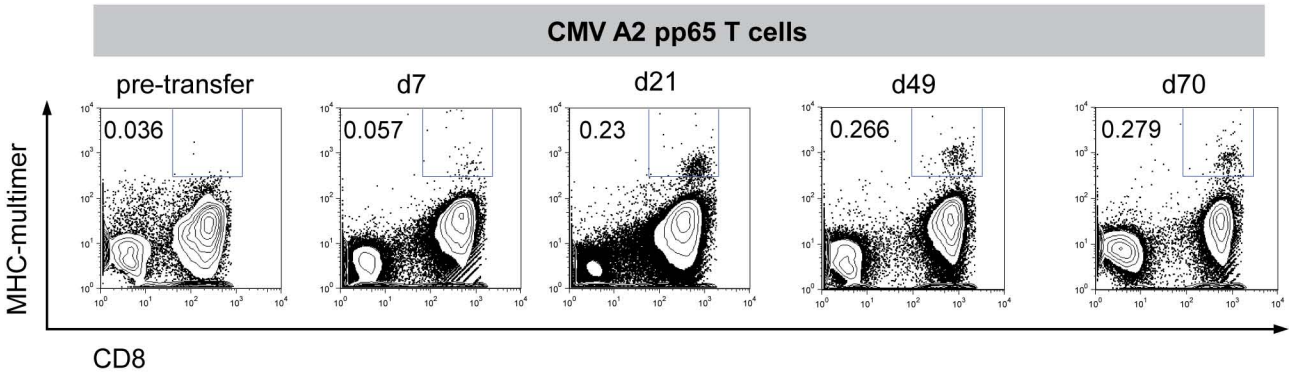
patient : Vβ13N ...TGTGCCAGC TTTTTT GGGGG TGGGG ATC...

donor : Vβ13N ...TGTGCCAGC TTTTTT GGGGG TGGGG ATC...

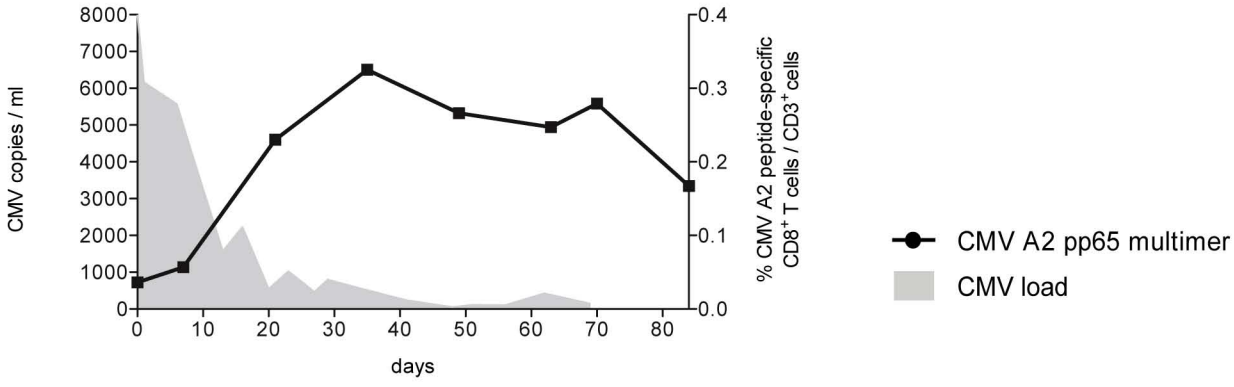
CDR3-specific primer

Fig. 5

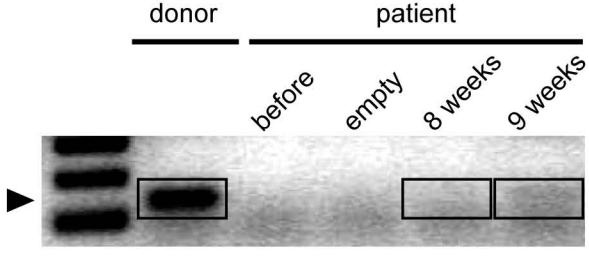
A



B



C



patient : Vβ13.1 ...TGTGCCAGC AGCCC CCAG ACCGGACTTC CTATGGCTACA...

donor : Vβ13.1 ...TGTGCCAGC AGCCC CCAG ACCGGACTTC CTA.....

CDR3-specific primer