Minor Histocompatibility Antigen (mHag)-specific And B-cell Chronic Lymphocytic Leukemia (CLL)-reactive T Cells Can Be Derived From Matched Sibling Donors Using Transformed CLL Cells As Stimulator Cells.

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Primary induction of allogeneic mHag-specific CLL-reactive T cell clones

Abstract

Objective: Allogeneic stem cell transplantation (SCT) following reduced-intensity conditioning provides new therapeutic opportunities for cure of advanced B-cell chronic lymphocytic leukemia (CLL). Although treatment of relapsed or persistent CLL after allogeneic SCT with donor lymphocyte infusions (DLI) can lead to complete sustained remissions, illustrating susceptibility of CLL for the graft-versus-leukemia (GvL) effect, effectiveness of this therapy is limited due to low immunogenicity of CLL cells and the lack of specificity resulting in concurrent development of graft-versus-host-disease (GvHD). To improve the specificity of the immune response, we investigate whether modified CLL cells are sufficiently immunogenic to initiate an adequate alloimmune response in vitro in a complete HLA-matched setting representing a clinical applicable transplantation model.

Methods: First, CLL cells were modified into efficient antigen-presenting cells (CLL-APC) by CD40 triggering in the presence of IL-4. Next, T cells derived from three unprimed HLA-matched sibling donors were stimulated with primary CLL or CLL-APCs from the patients.

Results: In contrast to primary CLL, CLL-APC cells as stimulators were capable of inducing cytotoxic CD8\(^+\) and CD4\(^+\) T-cell (CTL) clones from all three sibling donors. These CTL clones effectively lysed CLL-specific and patient-derived targets but not non-hematopoietic targets of the patient or donor-specific targets. The recognition of all clones was HLA-restricted and most likely mHag-specific.

Conclusion: In all three donor-recipient pairs tested, CLL-APC as stimulators were capable of inducing mHag-specific and CLL-reactive T cells clones. These results allow the development of new cellular immunotherapeutic interventions to further exploit the GvL effect following allogeneic SCT.
Primary induction of allogeneic mHag-specific CLL-reactive T cell clones

Introduction

B-cell chronic lymphocytic leukemia (CLL) is clinically and biologically heterogeneous \(^1,2\). Some patients may have an indolent course with long-term survival, whereas others have a rapidly fatal disease despite intensive therapy \(^1,2\). In addition to characteristic cytogenetic abnormalities, new molecular and protein markers such as the mutation status of the immunoglobulin variable heavy (VH) genes, expression of ZAP-70, and profiling of the expression of genes, enables to predict better patients, who will suffer from an aggressive and ultimately fatal course \(^4-8\). For this group of patients new effective treatment modalities are needed.

Recently, it has been demonstrated that allogeneic stem cell transplantation (SCT) may overcome the adverse prognosis of patients with CLL with unmutated VH genes \(^9\). Since allogeneic SCT following reduced-intensity conditioning (RIC) has resulted in lower short term treatment-related mortality (TRM) \(^10-14\) this treatment modality is increasingly being explored in patients with advanced CLL \(^11,15,16\). Donor T cells in the graft, recognizing leukemia-associated antigens or minor histocompatibility antigens (mHag) on CLL cells may initiate a robust alloimmune response and thus eradicate persisting or relapsing CLL in the recipient. Complete remissions have been reported after allogeneic SCT illustrating susceptibility of CLL cells to a graft-versus-leukemia (GvL) effect \(^11,15,17\). We and others demonstrated the feasibility of a two-step approach of allogeneic transplantation and immunotherapy following RIC by the use of T-cell depleted grafts avoiding the risk of graft-versus-host disease (GvHD), followed by a postponed administration of donor lymphocyte infusion (DLI) \(^13,18,19\). The approach was feasible even in older patients, but acute or chronic GvHD caused by the administration of donor lymphocytes remained a major cause of morbidity \(^13,20\). Furthermore, in some patients in vivo alloimmune responses sufficient to completely suppress the CLL cells could not be evoked. Therefore, further selection of donor T cells with high avidity for CLL cells, and further enrichment of leukemia-reactive donor T cells may be pivotal to achieve long-term control of the disease after allogeneic SCT.

Enrichment of donor T-cells for leukemia-reactive T-cell specificity may be achieved by in vitro stimulation of donor lymphocytes with leukemic cells from the patient \(^21,22\). This approach appears to select for T-cell responses against mHag that are relatively specifically expressed on hematopoietic cells \(^22\). However, although CLL cells highly express HLA class I and II molecules, they are inappropriate to function as antigen-presenting cells (APC) due to inadequate expression of costimulatory molecules \(^23-27\). We and others demonstrated that by CD40 triggering in the presence of IL4, CLL cells can be modified into efficient malignant APC, capable of inducing vigorous allogeneic T-cell responses \(^23,25-27\). CLL-reactive mHag specific cytotoxic T lymphocytes (CTL) derived from unrelated HLA class I-matched donors could be generated \(^27\).

To further translate these results in a clinically applicable transplantation model, and to investigate whether modified CLL cells are sufficiently immunogenic to initiate an adequate alloimmune response in a complete HLA-matched setting, T cells derived from three unprimed HLA-matched sibling donors
were stimulated with CLL-APCs from the patients. In all three donor-recipient pairs, CLL-APC as stimulators were capable of inducing mHag-specific and CLL-reactive T cells clones. These results support the potential of this approach to treat disease recurrence after allogeneic SCT.

**Material and methods**

**Cell samples**

Three patients treated for advanced CLL and eligible for allogeneic SCT using HLA-identical sibling donors were included in this study. After informed consent peripheral blood (PB) samples were obtained from the patients, and from their HLA-identical sibling donors. The HLA types of patients and donors are shown in Table 1. Mononuclear cells (MNC) were isolated from the samples by Ficoll density separation, and cryopreserved. As assessed by flow cytometry, more than 90% of the MNC from the CLL patients coexpressed CD19 and CD5 surface molecules. Phytohemagglutinin (PHA) activated T cells (PHA blasts) and stable transformed B cell lines (EBV-LCL) were generated as described previously. Mesenchymal stem cells (MSC) were derived from the bone marrow (BM) of one patient by culturing the adherent cells for several weeks on DMEM supplemented with L-alanyl-L-glutamine, sodium pyruvate, 1 mg/mL glucose, and pyridoxine (Gibco BRL), and 10% fetal bovine serum (FBS)

**Table 1. HLA types of patient/donor pairs.**

<table>
<thead>
<tr>
<th>Pair</th>
<th>HLA class I</th>
<th>HLA class II</th>
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<tbody>
<tr>
<td></td>
<td>A3 A31 B8 B35 Cw4 Cw7</td>
<td>DR1 DR3 DQ2 DQ5 DP2 DP3</td>
</tr>
<tr>
<td>Pair 1</td>
<td>A2* A68 B51 B53 Cw4 Cw1</td>
<td>DR4 DR1 DQ3 DQ6 DP2 DP4</td>
</tr>
<tr>
<td>Pair 2</td>
<td>A1 A2† B8 B35 Cw4 Cw7</td>
<td>DR3 4 DQ2 DQ5 DP2</td>
</tr>
</tbody>
</table>

* patient was negative for HA-1, donor was positive for HA-1. † patient was positive for HA-1, donor was negative for HA-1

**Modification of CLL cells into CLL-APC**

CLL cells were transformed into CLL-APC as recently described. Briefly, CLL cells were cocultured on Itk murine fibroblast cells transfected with the human CD40-ligand (tCD40L; kindly provided by Dr.C.van Kooten, Department of Nephrology, Leiden University Medical Center). The fibroblasts were irradiated (70 Gy), and seeded at a concentration of 1x10^5 cells/well in 24-well plates (Costar, Cambridge, MA, USA). CLL cells were added at a concentration of 1x10^6 cells/well in medium consisting of IMDM (BioWhittaker, Verviers, Belgium) with 10% human serum and IL-4 (500 U/mL, Schering-Plough, Amsterdam, The Netherlands). After 4 days of culture at 37°C in a 5% CO₂ humified atmosphere, the CLL cells were harvested and washed twice. Viable cells were counted using eosin exclusion, analyzed by flow cytometry, and used as stimulator cells.
Phenotypic analysis of the CLL-APC and the cytotoxic T cell lines and clones

To perform immunophenotyping of the CLL-APC and of the generated T cell lines and clones, mouse MoAbs conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or phycoerythrin cyanine 5 (PE-Cy5) were used. These MoAbs included FITC-conjugated antibodies specific for CD3, CD5, CD40, CD54, CD58, CD86, or HLA-DR, PE-conjugated antibodies specific for CD4, CD19, CD23, CD56, CD80 or CD83, and Cy5-conjugated anti-CD8. All MoAbs were purchased from Becton Dickinson (BD, San Jose, CA, USA) except for anti-CD40 and anti-CD58 (Serotec, Oxford, England), anti-CD54 (CLB, Amsterdam, the Netherlands), anti-CD8 and anti-CD83 (Caltag, Burlingame, AL, USA) and anti-CD80 (Immunotech, Marseille, France). PE-labeled tetrameric complexes of HA-1 peptide bound to HLA-A2 molecules were prepared and used as described previously. After labelling all cell samples were analyzed on a FACScan (BD).

Generation of CLL-reactive CTL lines and clones

MNC from the three healthy HLA matched sibling donors at a concentration of 0.5 x 10^6 cell/well in 24-well plates (Costar) were stimulated with irradiated CLL-APC at a responder/stimulator (R/S) ratio of 10:1. IL-2 (100 IU/mL, Chiron, Amsterdam, Netherlands) was added at day 6, and 2 days after each (re)stimulation. The T cell lines were restimulated with irradiated stimulator cells at the same R/S ratios at days 9, 16 and 23. T cells were harvested 4-5 days after the third or fourth stimulation for phenotypic analysis, and used as effectors in cytotoxicity assays. T cell clones were generated from CLL-APC reactive CTL lines by single cell / single well sorting using a FACS-Vantage flow cytometer (BD). To exclude dead cells the suspensions were counterstained with propidium iodide (PI; Sigma, St.Louis, USA) at a final concentration of 0.5 µg/ml immediately prior to cell sorting. PI negative cells within the lymphocyte gate were sorted, and plated as single cells per well (single cell/well sorting) into 96-well microtiter plates containing 100 µl of feeder mixture consisting of culture medium, IL-2 (100 IU/mL), phytohemagglutinin (PHA, 800 ng/mL, Murex Biotech Limited, Dartfort, UK) and 15 Gy-irradiated allogeneic feeder cells (5 x 10^6 /mL). Proliferating T cell clones were selected, and restimulated with feeder-mixture. From day 21 the clones were functionally analyzed. The nomenclature used for each T cell clone represents the patient number followed by clone number.

Analysis of cytotoxicity and cytokine production

To determine the cytotoxicity of the T cell lines and clones standard 4 hours and 10-16 hours 51Cr-release assays were performed as described previously. Primary CLL cells, CLL-APC cells, EBV-LCL or PHA blasts from patient or donor were used as target cells. T cell lines and clones showing more than 10% specific lysis of target cells were considered cytotoxic. To determine HLA class I- and II-restriction of the recognition of the target cells, blocking studies were performed in selected experiments. Target cells were incubated with α-HLA class I antibodies (W6/32) or α-HLA class II antibodies (PdV5.2) at final concentrations of 10 µg/mL for 30 minutes before effector cells added. A human MoAb specific for HLA-B8 (BVK5B10) was used to determine HLA-B8 restricted recognition of the targets by CTL clones derived from donor 3. For analysis of interferon (IFN) γ production by the T cells against MSC, 5,000 T cells were cocultured on a monolayer of 5,000 plated MSC in 96 wells flat-
bottom plates. After 24 hours, supernatants were harvested and the concentration of IFN-γ was measured by ELISA (CLB, Amsterdam, The Netherlands).

**T-cell receptor (TCR) analysis**

The T-cell receptor (TCR) α and β chains of the CTL clones were determined by polymerase chain reaction and sequencing, as previously described 29. The sequences of the TCRβ chains were named according to the nomenclature described by Arden et al 30.

**Results**

**Generation of CLL-reactive CTL lines and clones**

Our previous study had illustrated that CD40 activation in the presence of IL-4 is an effective tool to increase APC function of CLL cells 27. Four days of CD40 and IL-4 stimulation effectively transformed CLL cells, derived from the three patients in this study, into CLL-APC with high expression of the costimulatory molecules CD54, CD58, CD80 and CD86 and enhanced expression of CD83 (figure 1).

![Figure 1](image-url)

**Figure 1. Expression of costimulatory and adhesion molecules on primary CLL and CLL-APC derived from patient 2.** All costimulatory and adhesion molecules on the CLL cells were significantly upregulated by CD40 activation in the presence of IL-4. Primary CLL cells from patient 2 were cultured in 24 wells plate (1x10⁶) for 96 hours in the presence or absence of IL-4 and tCD40L. The expression of CD54, CD58, CD80, CD86, and CD83 was analyzed by flow cytometry. Mean fluorescence intensity ratio (MFIR) was calculated as MFI cells stained by a specific MoAb / MFI cells stained by isotype-control MoAb, only if the percentage of positive cells was >10%. Results are representative for all three patients.

These CLL-APC and primary CLL cells were used as stimulator cells for T cells from the HLA-identical sibling donors. In all three donor-patient pairs, no proliferation but a decrease of T cells was observed in response to primary CLL cells as stimulator cells. In contrast, using CLL-APC as stimulators, vigorous expansion of donor T cells was observed, resulting in a 6-10 fold increase of the T cell
population after three weekly stimulations. Experiments to generate CLL-reactive CTL lines were performed twice for each patient-donor combination. The CTL lines derived from donor 1 consisted of 84 ± 4% (mean ± SD) CD4+ T cells and of 12 ± 2% CD8+ T cells, and the T cell line derived from donor 2 consisted of 91 ± 2% CD4+ T cells and 4 ± 2% CD8+ T cells. Flow cytometric analysis of the CTL lines derived from donor 3 at day 21 showed a large population CD3-CD56+ cells (78 ± 4%) and only 11 ± 6% CD4+ T cells and 6 ± 3% CD8+ T cells. All T cell lines generated were examined for their ability to kill primary CLL and CLL-APC as well as patient- and donor-derived other hematopoietic targets (PHA-blasts and/or EBV-LCL). Due to very low (<1%) normal B-cell counts in the MNC of patient 1 and 2, no EBV-LCL from these two patients could be generated. As illustrated in figure 2, CLL-APCs induced T cell lines with cytolytic activity against CLL-APC and patient-derived hematopoietic targets in all donor-patient pairs. No cytotoxicity was observed against primary CLL. In our previous study we had demonstrated that primary CLL could adequately be recognized and killed by alloreactive donor-derived T cells 27. Therefore, to determine whether low precursor frequencies of mHag-specific and/or CLL-specific cytotoxic T cells within the predominantly CD4+ T cell and the CD56+ populations were responsible for the low cytolytic activity against CLL targets, and to select for these cytotoxic T cells, cloning experiments of the α-CLL and the α-CLL-APC CTL lines in the donor-patients pairs were performed.

Figure 2. Cytotoxicity of CTL lines, derived from the three HLA-identical sibling donors, generated against primary CLL cells or against CLL-APC cells. (A) Using primary CLL as stimulator cells, no cytotoxicity was observed in the first donor-recipient pair (1), reactivity against patient-derived PHA blasts was observed in the second couple (2), and reactivity against CLL-APC was observed in the third couple (3), as measured in a 4-hours 51Cr-release assay. (B) Stimulation with CLL-APC induced cytolytic civity against CLL-APC in all donors and cytotoxicity against patient-derived targets in donor 2 and 3. Results are representative for two experiments for each donor-recipient combination.
In donor-recipient combination 1, from the α-CLL-T cell line, 5% of the 1 cell/well clonal T cells showed proliferation. None of the 11 CD4+ and the 3 CD8+ T cell clones were cytotoxic. The plating efficiency in the donor-recipient pair 1, from the α-CLL-APC T cell line, was 11%, resulted in 31 proliferating clones. As shown in figure 3A 3 out of 8 CD8+ T cell clones and 4 out of 23 CD4+ T cell clones exerted cytotoxic activity against CLL-specific targets and not against other patient- and donor-derived targets. In the donor-recipient pair 2, from the α-CLL-T cell line, two proliferating CD8+ clones and 12 CD4+ clones were obtained (plating efficiency 3%) with one CD8+ T cell clone recognizing CLL-APC and PHA blasts from the patient but not primary CLL (data not shown).

A total of 110 proliferating clones were generated from the α-CLL-APC T cell line from donor-recipient pair 2. As illustrated in figure 3B, nine out of 48 CD8+ T cell clones and one out of 62 CD4+ T cell clones recognized CLL- and patient-specific but not donor-derived targets. Only two CTL clones (clone 2.57 and 2.89) show reactivity against primary CLL cells whereas the other clones recognized the CLL-APC and/or PHA blasts from the patient (figure 3B). Because the CTL clones generated were not highly effective in killing primary CLL cells and because we did not succeed to generate EBV-LCL from the patients, these CTL clones were not further analyzed for HLA-restriction.

In the third donor-patient combination 101 proliferating clones (72 CD4+ and 29 CD8+ T cell clones) were obtained from the α-CLL CTL line and none of these clones exhibited patient- or CLL-specific cytotoxicity. Single cell sorting of the α-CLL-APC CTL line resulted in high clonal expansion efficiency of 22% of the cells isolated. Four out of 208 T cell clones (112 CD4+ and 96 CD8+ T cell clones) were reactive against patient-derived and CLL-specific targets. These CD8+ CTL clones failed to stain with the HA-1 specific tetramer. As illustrated in Figure 3C, three clones (clone 3.76, 3.82 and 3.87) were highly cytotoxic against primary CLL (42 ± 11%, mean ± SD, n=3), CLL-APC (82 ± 10%), EBV-LCL of the patient (88 ± 12%) and not against donor-derived EBV-LCL (3 ± 1%) in a 16 hour 51Cr-release assay. Clone 3.62 exhibited reactivity against CLL-APC (lysis 47%) and EBV-LCL of the patient (68%) but not against primary CLL and donor-derived targets. Significant although lower cytotoxicity (38 ± 7%) was observed against PHA blasts (figure 3), indicating that the clones are mHag-specific and that the recognized mHag is not B lineage-restricted.
Figure 3. Cytotoxicity and specificity of CTL clones derived from HLA-matched sibling donors, generated against CLL-APC as stimulator cells. (A) In donor-patient pair 1 four CD4+ and three CD8+ CTL clones, recognizing CLL-specific targets but not patient- or donor-derived targets, were generated using CLL-APC as stimulators. (B) Out of 110 proliferating T cell clones, one CD4+ (clone 2.57) and 9 CD8+ CTL clones were obtained. Primary CLL as target was only recognized by clone 2.57 and clone 2.89, whereas CLL-APC and PHA blasts from the patient were recognized by most CTL clones. (C) Four CD8+ T cell clones out of 208 T cell clones showed cytotoxicity against primary CLL, CLL-APC and patient-derived targets and not against donor-derived EBV-LCL and PHA blasts. Clone 3.62 recognized CLL-APC and EBV-LCL and PHA blasts from the patient but not primary CLL cells whereas the other clones efficiently killed all CLL-specific and patient-specific targets and not donor-derived targets. All four clones stained negative with the HA-1 specific tetramer. Cytotoxicity was tested at E/T ratios of 10:1 and measured in a 10-16 hour $^{51}$Cr-release assay. The $^{51}$Cr-release assay was performed twice for each CTL clone. Results are representative for these experiments.
Figure 4. Characterization of the CLL-reactive clones derived from donor 3. (A) HLA class I-restriction of cytotoxicity. EBV-LCL of the patient were used as target cells at an E/T ratio of 10:1 in a 10-hour ⁵¹Cr-release assay and anti-HLA class I MoAb (W6/32) and anti-HLA class II MoAb (PdV5.2) were used to block recognition by the CTL clones. (B) Determination of the HLA class I-restricting element. To determine the HLA class I-restricting element, the CD8+ CTL clone 3.87 was tested for reactivity against a panel of EBV-LCL from unrelated individuals. The recognition of EBV-LCL targets by clone 3.87 was shown to be HLA-B8 restricted. mHag specificity was further illustrated by differential recognition of two individuals who were siblings, unrelated to the donor-recipient pair (see *). (C) Confirmation of HLA-B8 restricted recognition of the CTL clone 3.87 by blocking with anti-HLA B8 MoAb. (D) Hematopoietic-restricted recognition by the CLL-reactive CTL clone 3.87. Recognition of hematopoietic cells of the patient (EBV-LCL and PHA blasts), the non-hematopoietic mesenchymal stem cells (MSC) of the patient and EBV-LCL of the donor by the CTL clone 3.87 as measured by IFN-γ ELISA.
Primary induction of allogeneic mHag-specific CLL-reactive T cell clones

Cytolytic activity of the CTL clones could completely be abrogated using the α-HLA class I MoAb and not by α-HLA class II MoAb (figure 4A), confirming HLA class I restricted recognition. The TCRs of the CLL-reactive CTL clones were analyzed by PCR and showed that clone 3.76, 3.82 and 3.87 were identical (AV3 AV21 BV1). To further analyze the HLA class I-restricting element of these identical CTL clones, clone 3.87 was tested for reactivity against a panel of EBV-LCL from unrelated individuals that shared an HLA molecule with the recipient. As shown in figure 4B, the recognition of the targets was HLA-B8 restricted. To confirm HLA-B8 restricted recognition, a blocking study using α-HLA B8 MoAb (BVK5B10) was performed and showed no cytolytic activity of clone 3.87 in the presence of this MoAb (figure 4C). From patient 3, we were capable of culturing MSC from the BM of the patient. These cells were used as targets to determine the possible reactivity of the CLL-reactive T cells against non-hematopoietic tissues of the patient (figure 4D). Whereas the leukemia-reactive T cells produced significant amounts of IFN-γ against EBV-LCL (221 pg/mL) or PHA blasts (72 pg/mL) of the patient, no IFN-γ was produced after coculture on the MSC or against donor-derived EBV-LCL.

In conclusion, in contrast to primary CLL cells, CLL-APCs strongly induced a proliferative response of T cells from HLA-identical donors. In all CTL lines generated low precursor frequencies of CLL-reactive and mHag-specific T cells were present. Repetitive stimulation with CLL-APCs and single cell per well cloning enriched for these CLL-reactive T cells. In the third donor-patient pair the CLL-APCs were characterized to have induced mHag-specific HLA-B8 restricted CTL clones, highly effectively killing CLL-specific targets and not non-hematopoietic cells.

Discussion

Allogeneic SCT following RIC has resulted in lower TRM and may prolong survival in patients with advanced CLL. Although several studies have shown the susceptibility of CLL cells to a GvL effect relapses and/or progression of the disease after allogeneic SCT do occur indicating that a more efficient alloimmune anti CLL response is pivotal to achieve long-term remissions. Recently, we demonstrated the feasibility to generate CLL-reactive CTLs in an unrelated HLA class I-matched setting. Following these results this translational study was performed to evaluate whether T cells from HLA-identical sibling donors can be triggered to preferential kill CLL-specific targets, allowing application of these CTLs as adoptive immunotherapy to treat disease recurrence after allogeneic SCT.

It has been demonstrated that activation of the CLL cells by IL-4 and CD40 triggering is essential to overcome allogeneic T cell anergy towards primary CLL cells. In this study, no proliferative impulse of donor T cells was observed in response to primary CLL cells and repetitive stimulation with these malignant cells, expressing low levels of costimulatory molecules, failed to induce CLL- or mHag-specific T-cell responses. Only CLL-APC as target was recognized by the CTL line derived from donor 3. In contrast CLL-APC as stimulators induced proliferation of T cells in all three donor-patient combinations. These CTL lines showed enhanced cytotoxicity against patient-derived targets and CLL-APC. As illustrated in figure 2, the CTL lines showed no activity against the primary CLL. We
have demonstrated that primary CLL as target can be efficiently lysed by T cells, derived from HLA class I-matched donors. Therefore, to analyze whether low precursor frequencies of mHag-specific and/or CLL-specific cytotoxic T cells were present within the predominantly CD4+ T cell and the CD3- CD56+ populations single cell per well cloning was performed of the α-CLL and the α-CLL-APC CTL lines to identify cytotoxic T cells with reactivity against primary CLL and/or patient-derived targets.

Only one cytotoxic CTL clone was obtained from one of the three donor-α CLL T cell lines. In contrast, from each donor-α CLL-APC T cell line several CTL clones, recognizing CLL-specific and patient-derived targets, were generated (see figure 3). In accordance with the observed cytotoxicity of the α-CLL-APC CTL lines, the CTL clones generated, preferentially recognized CLL-APC as target. However, in addition several CTL clones (e.g. clone 1.28, clone 2.57, clone 2.89 and clone 3.76) showed cytotoxicity against primary CLL. Some clones were active against patient-derived hematopoietic targets and CLL-APC (clone 2.45, clone 2.105 and clone 3.62) and not against primary CLL. These CTL clones may be able to recognize the CLL cells, once these leukemic cells become activated or start dividing. One might speculate that for an adequate alloimmune reponse in patients with disease recurrence after allogeneic SCT, CTL clones with different specificities might be necessary to control the disease. Our results indicate that CLL-APC as stimulator cells are sufficient immunogenic to induce T-cell responses in HLA-matched sibling donors and enrich for precursor T cells with reactivity to CLL- and patient-specific targets. Three CTL clones, derived from donor 3 effectively lysed primary CLL as well as EBV-LCL from the patient. Further analysis of the specificity of these CTL clones showed that these clones were mHag-specific, recognized their targets in a HLA-B8 restricted manner and used the same TCR. These clones were not reactive to MSC, cultured from BM of the patient, suggesting that the recognized mHag is not widely expressed in non-hematopoietic tissue. Our study illustrates that CLL-APCs are capable of stimulating mHag-specific CLL-reactive T cells, present at low precursor frequencies in unprimed MNC of the donor. Although other patient-derived APC may also be capable of eliciting mHag-specific T-cell responses, we think that CLL-derived APC will likely skew the immune response toward recognition of CLL-specific antigens of mHags, highly expressed on the CLL cells thus leading to a more specific and efficient T-cell response.

Our two-step approach of T cell-depleted allogeneic SCT following RIC using alemtuzumab with its intrinsic anti-CLL activity followed by the postponed administration of DLI was shown to be feasible in CLL patients. With the data from our study several strategies to limit the occurrence of severe GvHD after adoptive immunotherapy while preserving and enhancing GvL activity can be considered. High doses of CLL-reactive and relatively hematopoiesis-restricted mHag-specific T cells or low dose DLI in combination with these leukemia-reactive T cells can be administered. In addition, CD8+ alloreactive T-cells are considered to be main effectors of GvHD and may not be necessary for the GvL reactivity.
Since only 5% of all T cell clones tested showed cytotoxicity against CLL-specific targets, our study clearly demonstrates that the use of extensive in vitro culture periods is not very efficient in selecting and isolating leukemia-specific T cells, is labor intensive. Early detection and isolation of leukemia-reactive T cells in the immune response using the IFN-γ capture assay may result in higher efficiency rates of CLL-reactive CTL clones 29,32.

In conclusion, this study illustrates that mHag-specific CLL-reactive CTL clones, derived from HLA-identical sibling donors, can be generated using CLL-APCs as stimulator cells. Recently, follow-up of a large cohort of patients with chemo-refractory CLL, treated with an allogeneic SCT following RIC, showed the feasibility of the transplant procedure, but also reported a relapse rate of 26% after 2 years 15. In this setting adoptive immunotherapy with donor-derived mHag-specific CTLs, which preferentially kill CLL cells and not non-hematopoietic targets could be of great value and could treat or even prevent disease recurrence after allogeneic SCT.
References

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