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Human Vγ9Vδ2 T Cells Specifically Recognize and Kill Acute Myeloid Leukemic Blasts

Julie Gertner-Dardenne,*†‡ Remy Castellano,§ Emilie Mamessier,*† Slaveia Garbit,§ Eloise Kochbati,*† Anne Etienne,§ Aude Charbonnier,§ Yves Collette,§ Norbert Vey,§ and Daniel Olive*†‡

Vγ9Vδ2 T cells are attractive candidates for antileukemic activity. The analysis of Vγ9Vδ2 T cells in newly diagnosed acute myeloid leukemia (AML) patients revealed that their absolute cell numbers were normal in the bone marrow but showed a striking imbalance in the differentiation subsets, with preponderance of the effector memory population. This unusual phenotype was restored after removal of leukemic cells in patients, which reached complete remission after chemotherapy, suggesting that leukemic cells might be involved in the alteration of γδ T cell development in AML. Accordingly, coculture between AML cells and Vγ9Vδ2 T cells induced selection of effector cells. In accordance with their effector memory status, in vitro proliferation of Vγ9Vδ2 T cells was reduced compared with normal controls. Nevertheless, Vγ9Vδ2 T cells efficiently killed autologous AML blasts via the perforin/granzyme pathway. The ligands for DNAM-1 were expressed by AML cells. We showed that killing of AML blasts was TCR and DNAM-1 dependent. Using a xenotransplantation murine model, we showed that Vγ9Vδ2 T cells homed to the bone marrow in close proximity of engrafted leukemic cells and enhanced survival. These data demonstrate that Vγ9Vδ2 T cells are endowed with the ability to interact with and eradicate AML blasts both in vitro and in a mouse model. Collectively, our data revealed that Vγ9Vδ2 T cells have a potent antileukemic activity provided that optimal activation is achieved, such as with synthetic TCR agonists. This study enhances the interest of these cells for therapeutic purposes such as AML treatment. The Journal of Immunology, 2012, 188: 4701–4708.

Compelling evidence has demonstrated the ability of the immune system to control acute myeloid leukemia (AML) following allogeneic stem cell transplantation and healthy donor lymphocyte infusions (1, 2). Unfortunately, the occurrence of graft-versus-host disease is a frequent and life-threatening complication (1, 2). Innate immunity also plays an important role in the AML control since allogeneic NK cells are involved in leukemia cell clearance as shown by the effect of killer cell inhibitory receptor-mismatched haploidentical stem cell transplantation (3).

In addition to conventional MHC class I-restricted CD8+ CTL and NK cells, unconventional T cells, including γδ T cells, might contribute to the immune defense against AML.

So far, T cells and NK cells are the major immune effectors shown to be involved in AML control at least in allogeneic settings. However, leukemic blasts induce immune suppression, which is likely responsible for the failure of immunotherapeutic approaches in the host. After transplantation of haploidentical hematopoietic stem cells and infusion of donor T cells, leukemic cells can escape from the donor’s antileukemic T cells through the loss of the mismatched HLA haplotype, leading to relapse (4).

In addition to conventional MHC class I-restricted CD8+ CTL and NK cells, other unconventional T cells, notably γδ T cells, display the same sensitivity and cytolytic power as NK and T cells. γδ T cells are involved in the immune defense against various leukemic cells (5–7) and notably chronic myeloid leukemia cells (8, 9), but their role in the specific control of AML remains unknown. Their antileukemic role was initially suggested by the observation showing that an increased reconstitution of donor Vδ1 T lymphocytes, posttransplantation, correlated with a better prognosis (5, 6). The Vδ1 T cell population is also predominant in newly diagnosed leukemia patients, suggesting that a γδ T cell-based immune response indeed occurs against primary leukemia (7). Another interesting subpopulation of γδ T cells is the γδ T cells bearing the Vγ9Vδ2 TCR. Vγ9Vδ2 T cells are the main subset of circulating γδ T cells, representing 1–10% of human peripheral T cells. They have the ability to simultaneously recognize and respond to phosphorylated nonpeptide Ags (phosphoantigens [PAgS]) (10), molecules expressed on cells undergoing neoplastic transformation (11) in an HLA-unrestricted fashion (12), and exert specific lysis of tumor cells (13). Vγ9Vδ2 T cells are thus involved in tumor immune surveillance (14–16), notably against epithelial cells carcinomas (17–21) and some hematopoietic.
etitic cells malignancies (8, 16, 22), but their involvement against AML has not been reported so far. Most importantly, because of their unique ability to respond to PAGs, we have found for >10 y how to easily amplify and activate Vγ9Vδ2 T cells ex vivo with the phosphorylated bromohydrin (BrHPP) analog that mimics the biological properties of natural PAGs (23). Whereas NK cell expansion remains tedious to achieve and has limited so far the feasibility of NK cell-based immunotherapy (24), Vγ9Vδ2 T cells, which can be specifically expanded in vivo (25), appear as ideal candidates for immune-based immunotherapy.

The aim of the study was to determine whether Vγ9Vδ2 T cells recognize AML blasts and help to control AML cell growth. In this study, we report that Vγ9Vδ2 T cells are mature effector cells able to interact in vivo and lyse AML blasts. We next identified which Vγ9Vδ2 TCRs and their ligands are responsible for triggering lysis of AML blasts. Finally, we illustrated the ability of ex vivo activated and expanded Vγ9Vδ2 T cells to control AML progression in an immunodeficient NOG mouse model of AML. Collectively, our data strongly suggest that Vγ9Vδ2 T cells are really attractive candidates for autologous ex vivo γδ T cell infusion, in the prospect of controlling residual AML leukemia.

**Materials and Methods**

**Reagents and Abs**

BrHPP was from Innate Pharma (Marseille, France). Recombinant human (rh)IL-2 and rhIL-15 were from BD Biosciences (San Jose, CA). The mAbs used for inhibition experiments and immunofluorescence analyses are listed in Supplemental Table I.

**Patients**

Twenty patients with AML entered this study after informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Sixteen of them were evaluated at diagnosis (16 of 16 were analyzed for their phenotype and 11 of 16 were evaluated for their proliferative capacity) and 4 after first complete remission following induction and consolidation chemotherapy remission. The study was approved by the Institutional Review Boards of the Institut Paoli-Calmettes. The control group consisted of nine age- and sex-matched healthy volunteers (HV) provided by Marseille Blood Bank. Human healthy bone marrow mononuclear cells (StemCell Technologies, Grenoble, France) were used as control for bone marrow mononuclear cells from AML. The mononuclear cells from AML patients and from HV were isolated by density gradient centrifugation (Lymphoprep; AbCys) and viably frozen in FBS (PAN Biotech) containing 10% dimethyl sulfoxide (Sigma-Aldrich) until use.

**Cell culture**

Effector γδ T cells were established and maintained as previously described (26) with minor modifications. In fact, because IL-15 was demonstrated to elicit survival and proliferation of γδ T cells (27, 28) throughout this study we have used the combination of IL-2 and IL-15 as described below. Briefly, PBMCs were stimulated BrHPP (3 μM), rhIL-2 (100 IU/ml), and rhIL-15 (10 ng/ml). rhIL-2 and rhIL-15 were renewed every 2 d at the same concentration and cells were kept at 1.5 × 10^6 cells/ml. The Burkitt’s lymphoma cell line Daudi and the AML cell line U937 were cultured from the American Type Culture Collection and were cultured (0.5 × 10^6 cells/ml) in complete RPMI 1640 medium with 10% FBS.

**Flow cytometry**

PBMCs (2 × 10^5) were washed in PBS (Cambrex Bio Science) and incubated at 4°C for 20 min with the specified mAbs. Cytotoxicity assays based γδ T lymphocyte expression of CD107a by lytic γδ T cells have been described elsewhere (26). For analysis of GranToxiLux (Oncoimmunin, Gaithersburg, MD), target cells, previously fluorescently labeled with TFL4, according to the manufacturer’s instructions, were incubated with granzyne B substrate with or without effector cells at a 5:1 E:T ratio for 1 h. Following incubation and washing, samples were analyzed on a FACS-Canto II using the DIVA software (BD Biosciences, San Jose, CA). Because γδ T lymphocytes capture membrane markers from the cells they interact with (26), the quantification of PKH67 transferred from cells previously stained with this dye provided a measure of γδ T cell trogocytosis to target cells in various conditions. The flow cytometry-based measure of T cell binding was calibrated by using FITC-coupled Quantum beads (Bang Laboratories, Fishers, IN) to convert the mean fluorescence intensities of PKH67 into the number of molecules of equivalent soluble fluorochrome (MESF); the increase from 0 to 60 min gave the transferred MESF data (29).

**Chromium release assay**

Target cells (1 × 10^6) were incubated with 100 μCi [51Cr] (Amersham, Buckinghamshire, U.K.) for 90 min and mixed with effecter cells in 150 μl RPMI 1640 medium with 10% FCS. After 4 h incubation at 37°C, 50 μl supernatant of each sample was transferred in Luma plates and radioactivity was determined by a gamma counter. The percentage of specific lysis was calculated using the standard formula [(experimental – spontaneous release)/(total – spontaneous release) × 100] and expressed as the mean of triplicate samples.

**Time-lapse confocal microscopy**

Visualization of perforin release at the single-cell level was performed using time-lapse confocal microscopy as previously described (26).

**NOG mouse/human AML model**

NOD-SCID common γ-chain knockout mice (NOG mice) were purchased from The Jackson Laboratory (Bar Harbor, ME). All animal procedures were performed in accordance with protocols approved by the local Committee for Animal Experiments. Healthy 6- to 8-wk-old female mice received a single i.v. injection of 0.2 × 10^6 U937 cells and were then randomly assigned to receive a single i.v. injection of 40 × 10^6 enriched Vγ9Vδ2 T cells (42% CD3+TCRVδ2+) or postexpansion-purified Vγ9Vδ2 T cells (98.8% CD3+TCRVδ2+; Unité de Thérapie Cellulaire et Génique de Nantes) on days 0 and 3. When leukemia-related symptoms were observed (hunchbacked, significant weight loss, and ruffled coat), mice were sacrificed. For in vivo imaging, Vγ9Vδ2 T cells were labeled with 3.5 μg/ml XenoLight DiR (Caliper Life Science, Hopkinton, MA) according to the manufacturer’s instructions and i.v. injected (10 × 10^6 cells/mouse) in mice xenografted with luciferase-transfected U937 for 14 d. The control group was injected with 10 × 10^6 cells per mouse in PBS. Mice were imaged with a Photon Imager (Biospace Lab, Paris, France) at different time points after injection.

**Statistical analysis**

The normality and variance of each sample series was evaluated before statistical analysis by the specified tests using α = 5% for significant differences. Briefly, a Mann–Whitney rank sum test was used for data shown in Figs. 1 and 4. A Wilcoxon matched-pairs test was used for data shown in Figs. 2 and 3. Comparison of survival curves in Fig. 5 was carried out with a Wilcoxon test. All statistical analyses were performed using GraphPad Prism 5 software.

**Results**

Vγ9Vδ2 T cells recognized and killed AML

A crucial question is to know whether Vγ9Vδ2 T cells were able to recognize AML blasts. We thus monitored Vγ9Vδ2 T cell interaction with AML blasts using the PKH67 transfer assay, which measures a cell contact-dependent uptake of PKH67 tracker by AML cells showing that Vγ9Vδ2 T cells captured 13,219 and 6,998 MESF, respectively, while γδ T cells isolated from HV (n = 9) captured 3,793 and 4,852 MESF. Vγ9Vδ2 T cells isolated from AML patients (n = 11) captured 12,904 and 6,695 MESF, respectively, showing that γδ T cells interact spontaneously to these cells. Notably this binding was significantly increased by PAG treatment (Fig. 1A, 1B).

We next sought to determine whether Vγ9Vδ2 T cells isolated from AML patients can be activated and expanded ex vivo. The stimulation was performed after in vitro stimulation with BrHPP plus IL-2 and IL-15 for 14 days to ensure maximal proliferation and cell survival (27, 28) (Fig. 1C). The proliferation of Vγ9Vδ2 T cells from AML (n = 11) was compared with the proliferation obtained with Vγ9Vδ2 T cells isolated from HV (n = 9).

Proliferation of Vγ9Vδ2 T cells from AML patients was strong but nonetheless slightly lower than the proliferation of Vγ9Vδ2
the presence (black bars) or absence (gray bars) of BrHPP.

confocal microscopy to visualize perforin release by Vγ9Vδ2 T cells. We next used time-lapse microscopy to visualize perforin release by Vγ9Vδ2 T cells, which inhibits the perforin-based cytotoxicity by increasing the pH of lytic granules, dramatically decreased AML blasts lysis (Fig. 2D). This result indicates that Vγ9Vδ2 T cells exert their cytotoxic activity against AML blasts through the perforin/granzyme pathway. Collectively, these results clearly identified AML as potential targets for Vγ9Vδ2 T cell recognition.

T cells from HV (expansion rate ranging between 17.7 and 193.7; see Supplemental Table II).

Finally, the interaction between Vγ9Vδ2 T cells and AML blasts resulted in the direct cytolysis of leukemic cells. Cytotoxicity was significantly increased after PAg stimulation of Vγ9Vδ2 T cells (Fig. 1D). These results demonstrated that Vγ9Vδ2 T cells could recognize, become activated, and kill AML blasts.

AML blasts are killed by expanded Vγ9Vδ2 T cells through a perforin/granzyme-dependent lysis

The main cytolytic pathway used by killer cells involves the release of cytolytic granules in effector/target synapse. Hence, we observed that 14 d-expanded Vγ9Vδ2 T cells from AML patients displayed a high degranulation potential toward autologous AML blast, equivalent to those of HV (Fig. 2A). We next used time-lapse confocal microscopy to visualize perforin release by Vγ9Vδ2 T cells. Expanded Vγ9Vδ2 T cells from HV were labeled with LysoTracker Red to trace their acidic perforin granules and co-incubated with primary AML blasts previously loaded with calcine AM (green) and coincubated with HV-expanded Vγ9Vδ2 T cells labeled with LysoTracker Red. Scale bar, 10 μm. (C) Representative experiment showing granzyme B (Gzm B) transfer into U937 cells mediated by resting or activated HV#1 Vγ9Vδ2 T cells. The assay was performed using the GranToxiLux assay as described in Materials and Methods. (D) Percentage of specific lysis of AML blasts by expanded Vγ9Vδ2 T from HV in a standard [\(^{51}\text{Cr}\)]-release assay by short concanamycin A (ConA) treatment. Wilcoxon paired test was used to compare differences between treatments: \(*p < 0.05, **p < 0.01, ***p < 0.001, p > 0.05\) is not shown.

FIGURE 2. Specific lysis of AML autologous primary blasts by expanded Vγ9Vδ2 T cells. (A) CD107a/b expression by expanded Vγ9Vδ2 T cells from HV (n = 9) and AML patients (n = 5) after 14 d culture with BrHPP and IL-2/IL-15 after stimulation with autologous blasts for AML and the same blasts for HV. (B) Time-lapse video confocal microscopy of AML blast loaded with calcine AM (green) and coincubated with HV-expanded Vγ9Vδ2 T cells labeled with LysoTracker Red. Scale bar, 10 μm. (C) Representative experiment showing granzyme B (Gzm B) transfer into U937 cells mediated by resting or activated HV#1 Vγ9Vδ2 T cell. The assay was performed using the GranToxiLux assay as described in Materials and Methods. (D) Percentage of specific lysis of AML blasts by expanded Vγ9Vδ2 T from HV in a standard [\(^{51}\text{Cr}\)]-release assay by short concanamycin A (ConA) treatment. Wilcoxon paired test was used to compare differences between treatments: \(*p < 0.05, **p < 0.01, ***p < 0.001, p > 0.05\) is not shown.

FIGURE 1. Vγ9Vδ2 T cells from AML were able to proliferate and to recognize leukemic blasts. (A) Representative experiment showing γδ T cell trogocytosis to AML blast from patient UPN03 before (full histogram) and after 1 h in coculture with (full line) or without BrHPP (dashed line). (B) γδ T cell trogocytosis (acquired MESF) to AML blast from 11 patients was obtained by subtracting the value at 0 min from that at 60 min. (C) PBMCs from HV (n = 9) and AML patients (n = 11) were cultured 14 d with 3 μM BrHPP plus 100 U/ml rhIL-2 and 15 μg/ml rhIL-15. Fold increase of viable Vγ9Vδ2 T cells was calculated at day 0 and 14 according to the formula: [(% γδD14 × total cell numbers at day 14)/(% γδD0 × total cell numbers at day 0)]. (D) Specific lysis of AML blasts by expanded Vγ9Vδ2 T cells from HV in a standard [\(^{51}\text{Cr}\)]-release assay in the presence (black bars) or absence (gray bars) of BrHPP.

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TCR and DNAM-1 ligands induced AML blast recognition and lysis from Vγ9Vδ2 T cells

Although the recognition of a target is primarily TCR-mediated, Vγ9Vδ2 T cells can also get activated by non–TCR-mediated pathways. So far, the receptors involved in the recognition of AML are unknown. Therefore, we analyzed the major activating receptors and coreceptors such as Vγ9Vδ2 TCR, NKG2D, and DNAM-1 and their ligands, known to be involved in Vγ9Vδ2
T cell activation. We observed a similar expression of NKG2D and DNAM-1 in Vγ9Vδ2 T cells from AML patients and from HV both ex vivo and after 14 d expansion with IL-2 and BrHPP (Table I and Supplemental Table III, respectively). Cell surface of the NKG2D ligands MICa and MICb was consistently negative in blasts of AML patients, whereas the levels of ULBP expression were either low or negligible (Fig. 3A). PVR and Nectin-2 were expressed at a higher frequency as compared with both ULBP and MICa/B expression (Fig. 3A). Interestingly, blasts from bone marrow (BM) (n = 8) and blood (n = 8) were not significantly different regarding NKG2D and DNAM-1 ligand expression (Fig. 3A).

To dissect the respective involvement of Vγ9Vδ2 TCR, NKG2D, and DNAM-1 in Vγ9Vδ2-mediated lysis, we performed cytolyis assays in the presence of blocking mAbs (Fig. 3B). Autologous AML blasts were used as target cells, and Daudi cells were used as a positive control (data not shown). As expected, mAb-mediated masking of TCR resulted in a drastic inhibition of target cell lysis. Masking of either DNAM-1 or its ligands (PVR and Nectin-2) resulted in a significant inhibition. In contrast, mAb-mediated masking of NKG2D did not reduce AML cell lysis. These data are in line with a strong expression of DNAM-1 ligands and a low or negligible expression of NKG2D ligands on AML blast. Collectively, these data shed light on the instrumental role of TCR and DNAM-1 as two key receptors involved in the recognition of AML blasts by Vγ9Vδ2 T cells.

**AML Vγ9Vδ2 T cells have a normal frequency, cytotoxic potential, and homing abilities as compared with HV but display a biased effector memory profile**

Because in vitro expansion rates of AML Vγ9Vδ2 T cells were found lower compared with HV γδ T cells, we hypothesized that subtle alteration in the phenotype or homing properties of γδ T cells occurred in patients. We analyzed the frequency and the phenotype of Vγ9Vδ2 T cells in the peripheral blood and BM of AML patients at diagnosis. CD3+Vγ9T cells were found in BM (n = 5, median 2.3% of CD3+ T cells) and blood (n = 5, median 2.2% of CD3+ T cells) of AML patients. These frequencies were similar to those obtained in age-matched HV (blood, n = 9; BM, n = 4). The major subset in AML patients consisted of Temh1 cells (53.8% in blood and 59.6% in BM). In contrast, the major subset in HV consisted of CM cells (47.3% in blood and 36.3% in BM) (Fig. 4A). This phenotype is consistent with the lower proliferative capacity of patients’ cells that we observed after in vitro stimulation (Fig. 1).

We searched for environmental effects as potential explanations for this differentiation pattern disparity. We observed no significant difference in the age of these two groups (64.3 ± 15 y for AML patients and 56.7 ± 3.5 y for HV) and no correlation was found between the proportion of Temh1 cells and any ongoing infection that might accelerate the Vγ9Vδ2 T cell differentiation process (presence or absence of fibrin; see Supplemental Table II).

To test whether the differentiation of Vγ9Vδ2 T cells into effector cells was induced after leukemic cell recognition, we coincubated PBMCs from HV with an increasing number of leukemic blasts. After 6 d, leukemic blasts induced a dose-dependent selection of the CD27+ subpopulation (effectors cells) among Vγ9Vδ2 T cells (Fig. 4B) without inducing apoptosis (data not shown). Moreover, normal naive and CM Vγ9Vδ2 T cell proportions (CD27+) were restored in a leukemia-free environment, that is, in patients undergoing remission after chemotherapy (Fig. 4C). Our observations suggest that recognition of leukemic blasts could induce the differentiation of Vγ9Vδ2 T cells into effector cells.

Collectively, these results converged to show that Vγ9Vδ2 T cells can specifically recognize and eliminate acute monocytic leukemia blasts in vitro, notably involving the TCR activation pathway. However, we did not exclude that malignant cells might modulate the efficiency of acute monocytic leukemia recognition, highlighting the potential benefit provided by the use of an agonist, such as BrHPP, to promote malignant cell eradication.

**Table I. Phenotypic analysis of Vγ9Vδ2 T cells from blood and BM of AML patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>γδ in T Cells</th>
<th>Per mm³</th>
<th>% Naives</th>
<th>CM</th>
<th>Temh1</th>
<th>TemRA</th>
<th>CD56</th>
<th>DNAM-1</th>
<th>NKG2D</th>
<th>CD158b</th>
<th>CCR5</th>
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<tr>
<td>UPN12 Blood</td>
<td>34.62</td>
<td>2.3</td>
<td>5.2</td>
<td>16.5</td>
<td>67.3</td>
<td>11.0</td>
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<td>62.1</td>
<td>90</td>
<td>8.5</td>
<td>32.5</td>
</tr>
<tr>
<td>UPN22 Blood</td>
<td>51.05</td>
<td>3.4</td>
<td>6.0</td>
<td>36.0</td>
<td>50.0</td>
<td>8.0</td>
<td>22.1</td>
<td>77.8</td>
<td>99.9</td>
<td>3.9</td>
<td>51.5</td>
</tr>
<tr>
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<td>3.1</td>
<td>10.8</td>
<td>37.8</td>
<td>43.2</td>
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<td>30.5</td>
<td>81.3</td>
<td>97.5</td>
<td>1.7</td>
<td>36.3</td>
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Results are expressed as percentage of positive cells gated on the TCRVγ9 population. *Data represent the median of the precised samples.
Successful antileukemic activity of Vγ9Vδ2 T cells in an in vivo model of AML

Our data strongly suggested that Vγ9Vδ2 T cells had a potent anti-AML activity in vitro. We next sought to validate these conclusions in a murine model. We developed a new xenotransplantation murine model to analyze the antileukemic activity of Vγ9Vδ2 T cells using NOG mice. This model is perfectly suitable to study cell interactions in vivo and allows high engraftment rates of human hematopoietic cells (31). We used the human AML cell line U937 that is sensitive to in vitro lysis by Vγ9Vδ2 T cells from AML patients or HV. Inoculation of U937 cells into healthy NOG mice resulted in development of hind leg paralysis and distress in all tested animals ~23 d after transplantation. Flow cytometry analysis showed that BM, spleen, and blood were infiltrated with U937 cells (Fig. 5A, left panel). We next coinfected BrHPP-treated PBMCs (42% of Vγ9Vδ2 T cells) and U937 cells to evaluate the distribution of Vγ9Vδ2 T cells in vivo in mice. After sacrifice, at day 17 after coinjection, Vγ9Vδ2 T cells were detected in BM, spleen, and blood of mice (Fig. 5A, right panel). The results indicate that this human AML NOG model is suitable for investigation of homing functions of Vγ9Vδ2 T cells.

For in vivo imaging of the Vγ9Vδ2 T cell homing process, Vγ9Vδ2 T cells highly purified after expansion with BrHPP (98.8% of purity) were stained with XenoLight DiR before being injected i.v. in NOG mice 14 d after luciferase-transfected U937 engraft. Noninvasive in vivo imaging showed the location of injected Vγ9Vδ2 T cells in close proximity to AML, notably in the BM, the original site of AML (Fig. 5B). Examination of organs harvested for ex vivo imaging 24 h postinjection confirmed the Vγ9Vδ2 T cells homing into the BM (Fig. 5C).

Next we examined the antileukemic activity of purified Vγ9Vδ2 T cells (98.8% of purity). Kaplan–Meier analysis of pooled cohorts of Vγ9Vδ2-treated (n = 10) and untreated mice (n = 10) reveals that mice receiving Vγ9Vδ2 T cells displayed a significant prolonged survival compared with untreated controls (p = 0.0243; Fig. 5D). These data demonstrated that human Vγ9Vδ2 T cells efficiently delay the development of the disease and exert a significant antileukemic activity in the adoptive NOG mouse transfer model.

Discussion

γδ T cells have been assigned as potent cells in antitumor immunity. In this study, we have characterized the phenotype and functions of Vγ9Vδ2 T cells and the requirements for these cells to conduct optimal antitumor responses. We report that γδ T cells from AML patients display disproportionate levels of effector memory cells compared with HV γδ T cells. This observation was correlated with a lower ex vivo expansion rate. In contrast, AML γδ
T cells did have a normal cell cytotoxicity capacity and did interact efficiently with AML blast cells both in allogeneic and autologous settings. Importantly, we showed that gd T cell-mediated killing of AML blasts was TCR and DNAM-1 mediated in a perforin/granzyme-dependent pathway. Finally, we set up a novel xenotransplantation model allowing us to demonstrate the potential of PAg-activated gd T cells in immunity against AML.

Analysis of gd T cell differentiation by means of the CD45RA and CD27 markers revealed a bias in the Vg9Vd2 T cell differentiation toward an effector phenotype. This subset is known to display higher functional capacities with an existing but reduced proliferation potential. Accordingly, we showed that gd T cells from patients had a reduced potential of expansion compared with their normal counterparts. Interestingly, we showed that AML blasts were involved in the skewing toward this effector memory phenotype. Indeed, in vitro interaction with blasts resulted in such skewing, and in patients removal of leukemic cells by chemotherapy resulted in the restoration of a normal differentiation pattern. Previously data obtained on ex vivo gd T cells from patients with multiple myeloma have revealed that gd T cell proliferation in response to zoledronate occurred in only 50% of patients. The effector memory phenotype CD45RA"CD27" was predominant in the multiple myeloma gd T cells that proliferated in response to zoledronate, whereas “late” effector cells (CD45RA"CD27") were predominant in those that failed to expand (32). The skewed effector profile observed here resembles the phenotype previously observed on αβ CD3"CD8" T cells from newly diagnosed AML (33). Although the authors did not evaluate the cytotoxic function of αβ CD3"CD8" T cells against autologous blasts, these cells showed the hallmarks of effector cytotoxic T cells (33).

Vg9Vd2 T cells are able to recognize tumor cells in a TCR-dependent manner (34), but some NK cell receptors are also expressed by Vg9Vd2 T cells, such as NKG2D and DNAM-1, which play an important role in the Vg9Vd2 T cell-mediated cytotoxicity against tumor cells (19, 20, 35). We have therefore analyzed the expression of ligands for these receptors. Except with the low expression of ULBP1, none of the NKG2D ligands was present on AML blasts. DNAM-1 ligands, PVR and Nectin-2, were expressed at the surface of leukemic cells. Consequently, we found that DNAM-1 was partly involved in the recognition and
killing of AML blasts. Similarly, DNAM-1 regulates the NK cell-mediated killing of several tumor types, including those of hematological origin (36–41). Recently DNAM-1 was implicated in the cytotoxicity of Vγ9Vδ2 T cells via a specific interaction with Necl-5 expressed by hepatocarcinoma cells (42). In contrast, we did not observe a significant implication of NKGD2, another major γδ TCR. Our data are in agreement with previous reports showing that NKGD2 plays a marginal role in AML killing by NK cells, especially in AML patients (40, 43). This was certainly due to the absence of NKGD ligand expression on AML cells. Conversely, the cytolytic activity of Vγ9Vδ2 T cells directly correlates with the surface expression of DNAM-1 ligands.

When studying the overall cytotoxicity potential of γδ T cells from AML patients, we found that these cells responded comparatively (with HV γδ T cells) well to AML blast interaction. This interaction was monitored both by trogocytosis (uptake of target cell material following cell/cell contact) and by direct cytotoxicity. However, we showed that PAg stimulation drastically increased such cell recognition. This points out the requirements for optimal response of γδ T cells in antitumor immunity and further strengthens the rationale for therapeutic use of ex vivo-expanded γδ T cells in immunotherapies for AML. This is further supported by a recent study showing that zolodeterminate treatment (another γδ T cell agonist) also improves γδ T cell functions in chronic myeloid leukemia (8).

Hence, we developed a preclinical mouse model by means of xenotransplantation of human leukemic cells together with γδ T cells. First, we found that infused γδ T cells colonized all organs tested, including the ones that are the tropism of AML in humans (i.e., BM and peripheral blood). Furthermore, mice coinfected with γδ T cells and AML cells had a higher survival probability compared with mice injected with AML cells alone. Thus, this model clearly established that γδ T cells could be a powerful tool for immune intervention. Recent studies have unraveled the role of γδ T cells in the response toward myeloid malignancies, such as chronic myeloid leukemia (8). However, our study reports for the first time the potential interest of γδ T cells in AML.

Nevertheless, we cannot exclude that the tumor might have acquired strategies to escape from or impede γδ T cell immunity. For instance, a recent study pinpointed an unknown inhibitory protein secreted by leukemic blasts that affects αβ T cell functions (44). Furthermore, in chronic lymphocytic leukemia, a study has shown that direct contact between the chronic lymphocytic leukemia tumor cells and the T cells induces both differential gene expression and defective formation of the immune synapse as compared with HV cells (45, 46). Hence, it is therefore conceivable that Vγ9Vδ2 T cells from AML patients actually encounter a certain inhibition from blast cells.

In conclusion, we have identified Vγ9Vδ2 T cells as potential mediators of innate antitumor immunity. We report in this study that appropriately stimulated γδ T cells are fully capable of activation and cytotoxicity in response to AML blast interaction. Killing of leukemic cells is achieved by means of TCR- and DNAM-1–dependent activation. This study point out the requirements for the use of γδ T cells in therapies against AML. Furthermore, such a targeted cell-based therapy would present the major advantage to be available for most AML patients, without adverse effects such as graft-versus-host disease.

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