Original Article

Expression of CD134 on CD4+ T cells reflects the immunosuppressive state after allo-HCT by revealing the intensity of T cell activation

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Abstract: The lack of reliable markers reflecting immune status after allogeneic hematopoietic stem cell transplantation (allo-HCT) inevitably results in either excessive or insufficient immune suppression, which undermines the post-HCT survival. The continuous activation of alloreactive T cells modulated by immunosuppressants provides clues to use the extent of T cell activation to evaluate immune status after allo-HCT. CD134 (OX40) is transiently expressed on activated T cells as a marker associated with recent activation; T cell activation plays an integral role in anti-infection, graft-versus-leukemia (GVL) effects and GVHD after allo-HCT. Accordingly, we evaluated whether the percentages of CD4+CD134+ T cell subsets could reflect immune status after allo-HCT in the context of GVHD, leukemic relapse and infection. Peripheral blood samples from 77 patients who underwent allo-HCT were subjected to flow cytometry analysis. The percentages of CD4+CD134+ T cells were significantly higher in patients with acute GVHD (aGVHD), moderate and severe chronic GVHD (cGVHD), Cytomegalovirus (CMV) infection and leukemic relapse compared with that in their control groups (P=0.006; P=0.011; P=0.002; P=0.018, respectively), thus it demonstrated that the percentages of CD4+CD134+ T cells could reflect T cell activation induced by GVHD, leukemic relapse and infection. We further observed that expression of CD134 on CD4+ T cells correlated with CMV clearance time (P=0.036), calcineurin inhibitors plasma concentration (P=0.004) and event free survival (EFS) rate of patients with leukemic relapse after allo-HCT (P=0.016). These results indicated that the percentages of CD4+CD134+ T cell could be the promising indicator for evaluating immunosuppressive status in patients after allo-HCT through reflecting the intensity of T cell activation.

Keywords: Graft versus host disease, allogeneic hematopoietic stem cell transplantation, CD134, T cell activation, calcineurin inhibitors

Introduction

allo-HCT has been a hitherto efficiently curative method for the malignant hematological diseases, but GVHD, infections and leukemic relapse are considered as 3 main risk factors that undermine the post-HCT survival [1-4]. The imbalanced immunosuppressive status after allo-HCT implies the etiology of such disorders. However, immunosuppressants are still empirically administered in clinical practice. The lack of reliable markers that reflect the extent of immune suppression inevitably results in either excessive immunosuppression which contributes to higher risk for infections and leukemic relapse or insufficient immunosuppression which increases GVHD risk. With the continuous stimulation by allo-antigens after allo-HCT, alloreactive T cells maintain persistent activated status which can be controlled by immunosuppressants. Therefore, the extent of T cell activation can theoretically reflect the extent of post-transplantation immunosuppressive status.

CD134 (OX40), a member of the tumor necrosis factor receptor family transiently (5 days) expresses on activated T cells as a marker associated with recent activation [5-7]. In vitro and vivo, expression of CD134 is markedly down-regulated by CNIs and glucocorticoids [8]. CD134 also functions as a co-stimulatory molecule by maintaining T cell survival and promoting cytokines production and plays an important role in the development and proliferation of memory T cell, especially in CD4+...
CD134+ CD4+ T cells and immune state after allo-HCT

Table 1. Patient characteristics

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<th>Patient characteristics</th>
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Abbreviations: AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; MAL: Acute mixed leukemia; CML: Chronic myelogenous leukemia; MDS: Myelodysplastic syndromes; AA: Aplastic anemia; CSA: Cyclosporine A; MTX: Methotrexate; MMF: Mycophenolate mofetil; ATG: Antithymocyteglobulin; Anti-CD25: Anti-CD25 antibody. aGVHD, non-aGVHD, MS-cGVHD, NM-cGVHD: Patients enrolled in those groups. CMV (+): Patients infected with CMV. Relapse: Patients with leukemic relapse after allo-HCT.

effector memory T cell [5-8]. CD134 is involved in pathogenesis of GVHD by interaction with its ligands as well [9, 10]. It was demonstrated that the percentages of CD4+ CD134+ T cells could predict the onset and therapeutic response of cGVHD [11-13]. T cell activation plays an integral role in anti-infection, GVL effects and GVHD in post-allo-HCT patients [11, 13-15].

Accordingly, we selected the percentages of CD134+ T cell subsets as the indicator for estimation of immunosuppressive status after allo-HCT and evaluated its feasibility in GVHD, infection and leukemic relapse settings.

Materials and methods

Study design and patients characteristics

Peripheral blood samples from 77 patients undergoing allo-HCT were collected in hematology department of Wuhan Union Hospital from Sep 1st 2014 to Feb 28th, 2015. All patients gave informed consent approved by the ethics committee of our institution before the procedure. Diagnosis of aGVHD was based on Seattle criteria [13]; cGVHD was diagnosed by 2005 NIH consensus criteria [3]. CMV infection was confirmed by detection of CMV-DNA ≥1000 copies/mL in plasma, while leukemic relapse was diagnosed as previously reported [16]. Patients were divided into different groups as follows: 1) aGVHD group and its control (non-aGVHD) group; 2) MS-cGVHD group (patients with moderate and severe cGVHD) and their control (NM-cGVHD) group (with 0 and mild cGVHD); 3) CMV (+) group (patients infected with CMV) and its control (CMV (-)) group, patients infected with CMV were subgrouped into ≥7 days group and <7 days group based on CMV clear time to evaluate anti-infection effects; 4) Relapse group (leukemia relapsed patients) and their control (non-Relapse) group, leukemia relapsed patients were further divided into two groups (with ≥20% or <20% CD4+ CD134+ T cells respectively) to evaluate GVL effects; 5) Patients with
alteration in administration of calcineurin inhibitors (CNIs) rather than other immunosuppressants were detected before and after tapering these medicines, and then were divided into Low IS group (with lower CNIs concentration) and High IS group (with higher CNIs concentration) based on plasma concentration of CNIs. The characteristics of patients were summarized in Table 1.

Samples from patients with GVHD were collected during the ongoing GVHD while samples from patients with CMV infection were tested on the day of onset of CMV-DNAemia. Samples from relapsed patients were detected right after diagnosis. Second detection of patients with adjustment of CNIs was performed during 7-14 days after tapering of CNIs. Patients with bacteria or fungal infection were ruled out by clinical manifestations and bone marrow aspiration in CMV (+) and CMV (-) group. GVHD and infections were excluded in Relapse and non-Relapse group. All relapsed patients discontinued CNIs before determination. Neither Low IS nor High IS group suffered infections or leukemic relapse within 2 weeks before determination.

Flow cytometry

Staining procedure was performed according to manufacturer’s instruction. 200 μl whole blood was collected from ethylenediaminetetraacetic acid (EDTA)-containing tubes. Briefly, 10 μL of anti-CD3 (FITC; Beckton Dickinson), 10 μL of anti-CD134 (PE; Beckton Dickinson), 10 μL of anti-CD4 (PerCP; Beckton Dickinson) and 10 μL of anti-CD8 (APC; Beckton Dickinson) were added in tubes above and the mixture was incubated at 4°C for 20 minutes. After red blood cell lysis, all samples were centrifuged and resuspended with PBS, then FACS Calibur (BD Bioscience) was used to analyze the subsets of CD134+ T cells.
CD134+ CD4+ T cells and immune state after allo-HCT

Statistical analysis

All data were analyzed with Graphpad Prism6.0, the normality of variables was estimated by Shapiro-Wilk tests before two-group comparison, Mann-Whitney U test and Wilcoxon matched-pairs test was used to analyze unpaired and paired variables without normality respectively; Analysis of variables with normality was done on Student t test or t test with welch’s correction according to the homogeneity of variances estimated by F test. Events free survival comparison was analyzed by Kaplan-Meier analysis. Differences with P <0.05 were considered to be statistically significant.

Results

The percentages of CD4+ CD134+ T cells were capable of reflecting T cell activation related to GVHD, CMV infection and leukemic relapse

Although CD134+ T cells were recognized as the recent activated T cells, whether the percentages of CD134+ T cell subsets could represent T cell activation induced by GVHD, CMV infection and leukemic relapse remains elusive, which was the prerequisite to use expression of CD134 on T cells to reflect post-transplantation immunosuppressive status. In GVHD settings, The percentages of CD3+ and CD4+ T cells rather than on CD8+ T cells significantly increased in aGVHD group compared with non-aGVHD group. D, E: Percentages of CD3+ and CD4+ CD134+ T cells were significantly different between I-aGVHD and non-aGVHD group but not between II-IV-aGVHD and non-cGVHD or I-aGVHD group. F: Percentages of CD8+ CD134+ T cells were comparable within 3 groups.

Figure 2. The percentages of CD134+ T cells in patients with acute GVHD were determined by flow cytometry. A-C: Expression of CD134 on both CD3+ and CD4+ T cells rather than on CD8+ T cells significantly increased in aGVHD group compared with non-aGVHD group. D, E: Percentages of CD3+ and CD4+ CD134+ T cells were significantly different between I-aGVHD and non-aGVHD group but not between II-IV-aGVHD and non-cGVHD or I-aGVHD group. F: Percentages of CD8+ CD134+ T cells were comparable within 3 groups.
Significantly elevated percentages of CD3+ CD134+ T cells and CD4+ CD134+ T cells were observed in aGVHD group ([14.78±1.80%] and [30.77±2.67%] n=13, respectively) than that in non-aGVHD group ([8.91±1.57%] and [19.72±2.54%] n=14, respectively) (P=0.021, P=0.006, respectively) (Figure 2A, 2B) while the percentages of CD8+ CD134+ T cells in aGVHD group were comparable to that in non-aGVHD group (P=0.188) (Figure 2C). In addition, significantly higher percentages of CD4+ CD134+ T cells and CD3+ CD134+ T cells were discovered in patients with leaGVHD ([31.28±3.63%] and [14.70±2.30%] n=9, respectively) than non-leaGVHD group ([19.72±2.54%] and [8.91±1.57%] n=14, respectively) (P=0.014 , P=0.043, respectively) (Figure 2E, 2D), but the percentages of CD8+ CD134+ T cells did not correlate with I-aGVHD (P=0.147) (Figure 2F).

The expression of CD134 on CD3+, CD4+, CD8+ T cell subpopulations were not significant different between patients with grade IaGVHD and grade II-IvaGVHD (P=0.950, P=0.787, P=0.494, respectively) (Figure 2F).

Since vivo T cell depletion in patients undergoing unmanipulated haploidentical hematopoietic stem cell transplantation, patients with T cell counts over 300/μl were enrolled to make sure detectable number of CMV specifically activated T cells. CMV (+) group presented significantly higher percentages of CD4+ CD134+ T cells [36.44±4.42%, n=4] (P=0.002) than CMV (-) group [15.46±1.16%, n=10] (Figure 3B) while The percentages of CD3+ CD134+ and CD8+ CD134+ T cells in CMV (+) group paralleled with that in CMV (-) group (P=0.380 and P=0.490, respectively) (Figure 3A, 3C).

Donor T cells activated by leukemic and allo-geneic antigens in relapsed patients mediated predominant GVL effects by killing leukemic cells [15]. Significantly higher percentages of CD4+ CD134+ T cells were detected in Relapse group [27.11±5.02%, n=7] than non-Relapse group [15.37±1.98%, n=13] (P=0.018) (Figure 3E), while neither the percentages of CD3+ CD134+ nor that of CD8+ CD134+ T cells showed significant difference between two groups.
CD134+ CD4+ T cells and immune state after allo-HCT

The percentages of CD4+ CD134+ T cells were capable of evaluating post-transplantation immunosuppressive state by reflecting anti-infections and GVL effects and correlation with plasma concentration of CNIs.

We further analyzed the percentages of CD134+ T cell subsets in the patients infected with CMV (including patients with GVHD and T cell counts <300/μL and CMV (+) group but excluding other infections and leukemic relapse). Significantly increased expression of CD134 on CD4+ T cells [40.14±5.15%, n=5] were discovered in <7 days group than that in ≥7 days group [8.04±8.04%, n=3] (P=0.036) (Figure 3A).

The intensity of GVL effects was associated with clinical outcome of relapsed patients [2]. To assess whether the percentages of CD4+ CD134+ T cells could reflect GVL effects, we further compared the EFS rate in two different T cell activation groups. We found that EFS rate within 3 months (0%, 0/5) was significantly lower in patients with <20% of CD4+ CD134+ T cells than that (75%, 3/4) in patients with ≥20% of CD4+ CD134+ T cells (P=0.016) (Figure 4B).

CNIs were widely administered in post-transplant patients to induce immunosuppression by inhibiting T cell activation and proliferation [17]. The correlation between the percentages of CD134+ T cell subsets and plasma concentration of CNIs was assessed in post-transplant patients. Significantly higher percentages of CD3+ CD134+, CD4+ CD134+ and CD8+ CD134+ T cells were observed in Low IS group compared with those in High IS group (P=0.041, P=0.004 and P=0.008, respectively) (Figure 4C-E).

Discussion

The present study shed some light on the feasibility associated with the percentages of CD4+ CD134+ T cells and immune state after allo-HCT.
CD134+ T cells to be the indicator of immunosuppressive status after transplantation. At first, we determined whether the percentages of CD134+ T cell subsets can reflect T cell activation induced by GVHD, CMV infection and leukemic relapse. Using muti-color flow cytometry, we showed that the percentages of CD4+ CD134+ T cells rather than CD8+ CD134+ T cells or CD3+ CD134+ T cells significantly increased in the context of GVHD, CMV infection and leukemic relapse after allo-HCT. We further demonstrated that the percentages of CD4+ CD134+ T cells negatively correlated with CMV clearance time and plasma concentration of CNIs but positively correlated with EFS rate of leukemic relapsed patients. These results demonstrated that the percentages of CD4+ CD134+ T cells at least partially represented the extent of T cell activation in post-transplant patients and subsequently reflected immune status after allo-HCT.

CD134 was characterized by its negative baseline and rapid up-regulation on both naive and memory T cells after stimulated by antigens and markedly down-regulated by CNIs and glucocorticoids [5, 6, 18]. CD134 was superior to other T cell activation markers such as CD69, HLA-DR, CD25, CD54 and CD137 [11, 13]. CD4+ CD134+ T cell played an important role in the development of GVHD. In transplantation models, transfusing OX40-deficient naive T cells to recipients markedly reduced GVHD onset and severity compared with mice accepted normal allogeneic naive T cells [10]. Although accumulated evidence suggested that significantly increased percentages of CD134+ T cell subsets in acute or chronic GVHD settings, the definite correlation remain controversial. Expression of T cell activation antigen CD134 (OX40) was not predictive for the occurrence or therapeutic response of aGVHD in partial T cell-depleted bone marrow transplantation [19]. In contrast, Ai Kotani and Takayuki Ishikawa et al reported the percentages of CD4+ CD134+ T cells could predict both the onset and response to therapy of aGVHD in non-T cell depleted HCT [11]. However, M. Paz Morante and J. Briones et al demonstrated that the percentages of CD4+ CD134+ T cells could only predict the therapeutic response rather than the onset of aGVHD in both patients with T cell depleted and non-T cell depleted HCT [13]. In aGVHD settings, our study was consistent with reported data, which showed aGVHD group presented higher percentages of CD4+ CD134+ T cells than non-aGVHD group. Whereas, our data suggested that only MS-cGVHD group, which usually need clinical intervention, had higher percentages of CD4+ CD134+ T cells than NM-cGVHD and mild-cGVHD group. However, neither the reported data nor our study revealed the correlation between expression of CD134 on T cells and severity of acute GVHD.

CMV infection was one of frequent complications resulting from compromised immune system in the early stage after allo-HCT [1, 20]. CMV induced T cell activation was related to initiation of anti-CMV cell immunity, especially CD4+ T cell activation. It was demonstrated that CD4+ T-helper cells regenerated relatively slowly after allo-HCT resulting in limited cytokines production, such as IL-2 and IFN-γ required by CD8+ T cells to eliminate CMV infected cells [21, 22]. CD4+ T cells co-expressed CD25 and CD134 induced by CMV antigens were CMV-specific [23]. Despite compromised immune response, T cells were still activated by CMV as we detected significantly increased expression of CD134 on CD4+ T cells in CMV (+) group. In addition, CMV clearance time was also demonstrated to be negatively correlated with the percentages of CD4+ CD134+ T cells. Efficient immune response targeting on CMV infected cells results in expeditious elimination of CMV. Therefore, the percentages of CD4+ CD134+ T cells were able to reflect immunosuppressive status by indicating anti-CMV capacity of immune system in the context of CMV infection. Persistent CMV-DNAemia could increase the risk of CMV end organ diseases with subsequent unfavorable outcome [24]. Evaluation of immune status in CMV infected patients, particularly those with persistent CMV-DNAemia, could provide vital clues to regulate immunosuppressants such as CNIs and preemptive transfusion of CMV specific T cells to enhance anti-infection effects.

Leukemic relapse still remains the major cause of death following allo-HCT [2]. GVL effects constitutes predominant therapeutic effects of allo-HCT through immunologic mechanisms such as donor T cells and NK cells responses [2, 25]. Donor T cells were to a large extent responsible for GVL effects. Leukemic antigens
or alloantigens on leukemic cells were firstly uptaken, processed then presented to T cells by dendritic cells. The ensuing T cells activation were detected in our study: significantly higher percentages of CD4+ CD134+ T cells was discovered in Relapse group. Donor lymphocyte infusion (DLI) was one of the most common methods to induce remission after relapse by intensifying GVL effects [14, 15]. However, with clonal evolution in relapsed leukemic cells, GVL effects might be slashed by genetic or epigenetic alterations associated with immune escape [26]. It was necessary to reconsider the role of DLI in regimen for relapse in this context for its limited GVL effects but overwhelmed GVHD, and highlight the assessment of GVL effects. GVL effects were considered to be associated with the clinical outcome of relapsed patients. Our data showed that patients with more intensive T cell activation achieved better EFS rate compared with patients with less intensive T cell activation. Thus the extent of T cell activation revealed by expression of CD134 on CD4+ T cells could reflect GVL effects in relapsed patients. The intensity of GVL effects should be evaluated to stratify relapsed patients and optimize the management of post-transplant relapse. For example, relapsed patients with limited GVL effects might be given more intensive chemotherapy or secondary HCT while patients with relatively intensive GVL effects are susceptible to regimen with DLI and chemotherapy. Further studies including clinical trials and cohort studies are needed to confirm such results.

Although CD134 expressed on CD4+ T cells was reported to be well controlled by CNIs and glucocorticoids both in vitro and in vivo, the suppressive effects of CNIs on expression of CD134 on CD4+ T cells after allo-HCT remains elusive. We detected elevated percentages of CD4+ CD134+ T cells after tapering CNIs, but the suppressive effects varied in different individuals as a result of different disparities of HLA, concomitant synergic medicines and sensitivity to CNIs. We concluded that plasma concentration of CNIs correlated to their immunosuppressive effects could also be reflected by the percentages of CD4+ CD134+ T cells. Accordingly, expression of CD134 on CD4+ T cells could be used as an indicator of immunosuppressive status to guide the administration of CNIs after allo-HCT. However, the definite percentages of CD4+ CD134+ T cells representing optimal immunosuppressive status defined as minimal GVHD but maximal GVL and anti-infection effects has yet to be explored.

In conclusion, our study firstly demonstrated the feasibility to use the extent of T cell activation reflected by expression of CD134 on CD4+ T cells to represent immune status after allo-HCT. With establishment of the indicator of post-transplant immune status, immunologic interventions tend to be more flexible and well directed in different situations after allo-HCT. However, more deliberately designed studies are needed to establish detailed stratification in relapsed settings and optimal immunosuppressive status after allo-HCT based on the percentages of CD4+ CD134+ T cells.

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Disclosure of conflict of interest

None.

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References


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