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LETTER TO THE EDITOR Open Access

Memory T cells skew toward terminal differentiation in the CD8+ T cell population in patients with acute myeloid leukemia

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Abstract

Stem cell memory T (T_{SCM}) and central memory T (T_{CM}) cells can rapidly differentiate into effector memory (T_{EM}) and terminal effector (T_{EF}) T cells, and have the most potential for immunotherapy. In this study, we found that the frequency of T_{SCM} and T_{CM} cells in the CD8+ population dramatically decreased together with increases in T_{EM} and T_{EF} cells, particularly in younger patients with acute myeloid leukemia (AML) (< 60 years). These alterations persisted in patients who achieved complete remission after chemotherapy. The decrease in T_{SCM} and T_{CM} together with the increase in differentiated T_{EM} and T_{EF} subsets in CD8+ T cells may explain the reduced T cell response and subdued anti-leukemia capacity in AML patients.

Keywords: Stem cell memory T cells, Central memory T cells, Effector memory T cells, CD8+ T cells, Acute myeloid leukemia, Bone marrow, Peripheral blood

To the editor

Clinical applications of immunotherapy for AML lag behind those for solid tumors and lymphocytic leukemia [1–3]. Recently, a new memory T cell subset, stem cell memory T (T_{SCM}), which has stem cell-like capacity, has been discovered [4–6]. However, little is known about the role of these cells in AML. In this study, we assessed the distribution of CD4+ and CD8+ T_{SCM}, central memory T (T_{CM}), T effector memory (T_{EM}), and T terminal effector (T_{EF}) cells in peripheral blood (PB) and bone marrow (BM) from patients with AML and those with AML in complete remission (AML-CR) by multicolor flow cytometry. The gating strategy used in this study followed a published protocol [7]. The CD4+ and CD8+ T cells were divided into four subgroups according to the CCR7 and CD45RO expression pattern: naïve and T_{SCM} cells (CCR7+CD45RO−), T_{CM} cells (CCR7+CD45RO+), T_{EM} cells (CCR7−CD45RO+), and T_{EF} cells (CCR7−CD45RO−). The T_{SCM} population was defined by double positive CD95 and CD28 expression.

The percentages of the T_{SCM}, T_{CM}, T_{EM}, and T_{EF} cells in the CD4+ and CD8+ populations were analyzed in 20 cases with AML (17 cases in newly diagnosed and 3 cases with AML relapse) (Fig. 1a, d) [8, 9]. The CD8+ T_{SCM} and CD8+ T_{CM} cells significantly decreased in the PB of these patients (Fig. 1e, g), whereas there was no significant change in the CD4+ population (Fig. 1b, g). Thus, the changes in the memory T cell subsets appeared to mainly involve CD8+ T cells. The shift from T_{SCM} and T_{CM} cells to a higher ratio of differentiated T_{EM} and T_{EF} cells is thought to be due to the constant exposure of T cells to AML cells and the leukemia environment, leading to T cell exhaustion and/or dysfunction [3].

To study the influence of the tumor microenvironment on the memory T cell distribution and function in leukemia patients, we collected seven pairs of PB and BM samples from AML patients at the time of diagnosis

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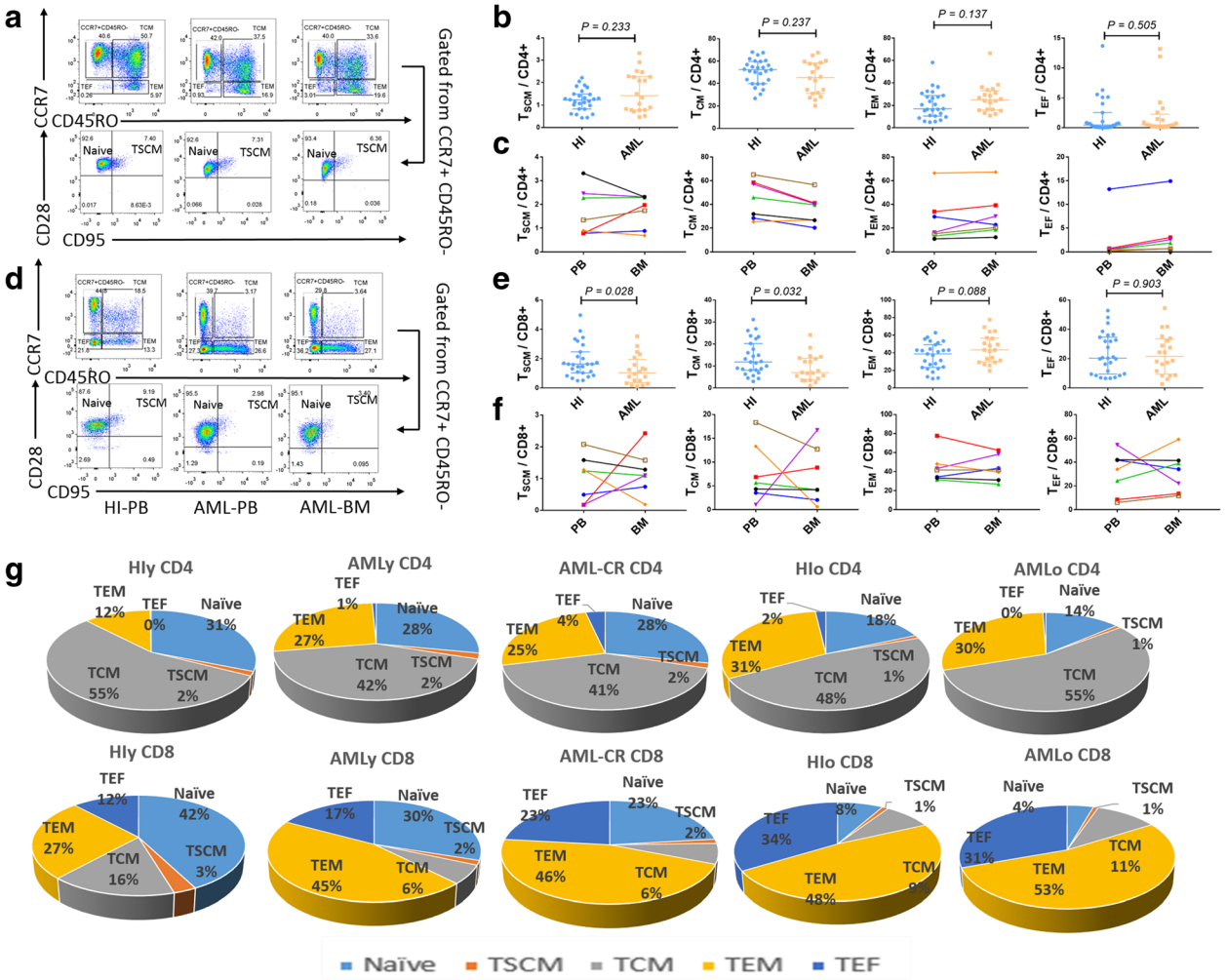

Fig. 1 Gating strategy for identifying the CD4+ and CD8+ T cells and the percentage of memory T cell subsets in the patients with AML and healthy individuals. **a**, **d** CD4+ (**a**) and CD8+ T (**d**) cells were differentiated into four subsets based on the expression of CCR7 and CD45RO in one HI-PB, one AML-PB, and one AML-BM patient: central memory T cells (CCR7+CD45RO+), effector memory T cells (CCR7−CD45RO+), and effector T cells (CCR7−CD45RO−). In the CCR7+CD45RO− subset, the expression of CD28 and CD95 was used to identify naïve T cells (CD28+CD95−) and T_{SCM} cells (CD28+CD95+). **b**, **e** Frequency of the T_{SCM}, T_{CM}, T_{EM}, and T_{EF} subsets in the CD4+ (**b**) and CD8+ (**e**) T cell populations from 27 HIs and 20 AML patients. **c**, **f** The subsets within the CD4+ (**c**) and CD8+ (**f**) T cell populations from PB and matched BM from seven AML patients, including different AML subtypes (M1, M2, M2b, M3, and M5), were compared. **g** Summary of the altered distributions within the CD4 and CD8 naive and memory T cell subsets in the AMLy, AMLo, and AML-CR groups compared with HIs. HIy ($n = 13$), AMLy ($n = 10$), AML-CR ($n = 9$), HIo ($n = 14$), AMLo ($n = 10$). HIs, healthy individuals; AML, acute myeloid leukemia; AML-CR, AML patients who achieved complete remission; PB, peripheral blood; BM, bone marrow; y, younger than 60 years; and o, older than 60 years. The differences in the different T cell populations in each of the T cell subsets were tested by two independent-sample Wilcoxon tests. Medians were calculated to represent all of the data. *P* values < 0.05 were considered statistically significant

and compared the distributions of memory T cell subsets. The differences in each subset appeared to vary widely (Fig. 1c, f). A low percentage of CD4+ T_{CM} cells and a corresponding high percentage of CD4+ T_{EM} and T_{EF} cells were observed in the BM compared with PB (Fig. 1c). In the CD8+ population, the changes appeared to be specific to each individual, and lower CD8+ T_{SCM} and CD8+ T_{CM} percentages were observed in the BM in half of the patients, whereas there were high percentages of CD8+ T_{SCM} and CD8+ T_{CM} cells in the BM compared with PB in the remaining samples. It has been reported that T cells in normal BM mainly possess a memory phenotype, particularly for CD8+ T_{CM} cells [10], suggesting that alterations in the leukemic BM niche in different AML individuals and AML subtypes may have different impact on T_{CM} homing.

Next, we compared the distribution of memory T cells in AML patients younger (AMLy) and older (AMLo) than 60 years [11]. Unlike healthy individuals (HIs), the memory T cell subset distribution in the AMLy cohort

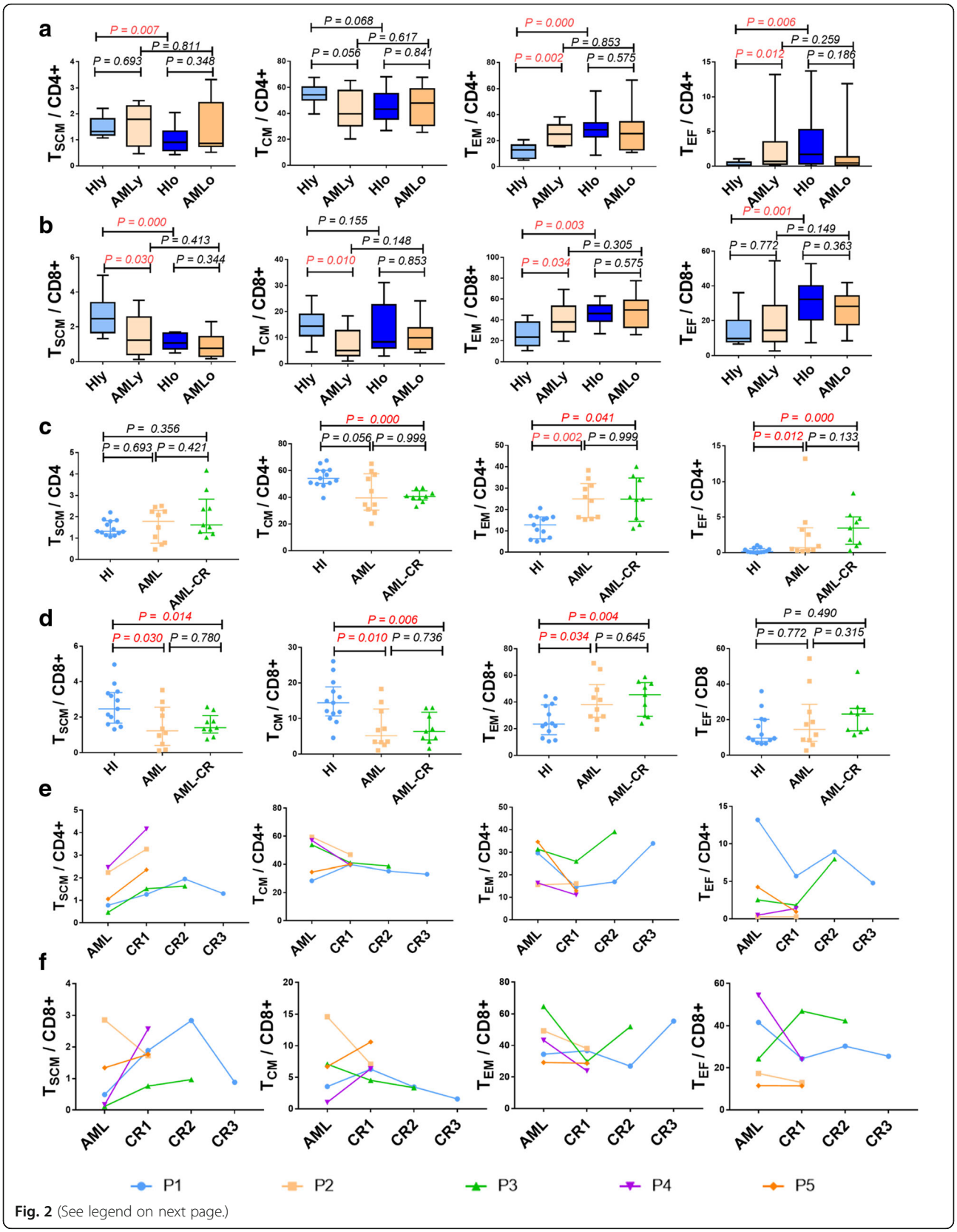

Fig. 2 (See legend on next page.)

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Fig. 2 Memory T cell subset distribution in CD4+ and CD8+ T cells in patients younger or older than 60 years with AML and AML-CR. **a**, **b** T_{SCM}, T_{CM}, T_{EM}, and T_{EF} subsets within the CD4+ (**a**) and CD8+ (**b**) populations in the HIy, AMLy, HIo, and AMLo groups. HIy ($n = 13$), AMLy ($n = 10$), HIo ($n = 14$), and AMLo ($n = 10$). **c**, **d**: Frequency of T_{SCM}, T_{CM}, T_{EM}, and T_{EF} cells within the CD4+ (**c**) and CD8+ (**d**) T cell populations in age matched HI, AML and AML-CR cohorts. HIs ($n = 13$), AML ($n = 10$), and AML-CR ($n = 10$). **e**, **f** Five AML patients were dynamically assayed for the T_{SCM}, T_{CM}, T_{EM}, and T_{EF} subsets in the CD4+ (**e**) and CD8+ (**f**) T cell populations at different time points. AML-CR, AML patients who achieved complete remission; P, patient; CR1, 2, 3, indicate different time points at which the patient achieved CR

was strikingly different than that in younger HIs (HIy) and tended to have a similar distribution pattern as that detected in the HIo and AMLo groups with a more obvious difference in the CD8+ population (Figs. 1g and 2a, b). These findings indicate that the leukemia microenvironment might drive T cell differentiation in AMLy. Whether such a skewed T cell distribution in AMLy truly represents T cell senescence remains an open question [8]; however, T cells in AMLo patients may not be able to further differentiate due to inherent T cell senescence, which may be an immune factor underlying the inferior prognosis of AMLo patients. Together, these data may suggest that T cell exhaustion and senescence are involved in T cell immune impairment, leading to an inefficient anti-tumor response.

We next compared differences in the distribution of memory T cell subsets between the AMLy, AML-CR, and HIy groups. A persistent, skewed memory T cell distribution was demonstrated for AML patients who achieved CR after chemotherapy (Fig. 2c, d). CD4+ and CD8+ T_{SCM} cells were predominantly increased at different time points after CR, while the change in other memory T cell subsets was relatively different (Fig. 2e, f). Overall, with the exception of incomplete recovery of the T_{SCM} cells, the reduction in T_{CM} cells and corresponding excessive accumulation of T_{EM} and T_{EF} cells were more evident in AML patients with CR (Fig. 1g), which may be related to the immune suppression of chemotherapy.

Abbreviations
AML: Acute myeloid leukemia; BM: Bone marrow; CML: Chronic myeloid leukemia; CR: Complete remission; HSCT: Hematopoietic stem cell transplantation; PB: Peripheral blood; PBMCs: Peripheral blood mononuclear cells; T_{CM}: Central memory T cells; T_{EF}: Terminal effector T cells; T_{EM}: Effector memory T cells; T_{SCM}: Stem cell memory T cells

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
YQL contributed to the concept development and study design. LX coordinated the study. LX, DLY, JXT, ZFH, SHL, XFZ, and SHC performed the laboratory studies. ZY, JC, GXL, CLW, and FFZ collected the clinical data. DLY contributed to figure preparation. YQL, XL, and DLY drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This study was approved by the ethics committee of The First Affiliated Hospital of Jinan University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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