Enhancement of the TCR ζ Expression, Polyclonal Expansion, and Activation of T Cells from Patients with Acute Myeloid Leukemia After IL-2, IL-7, and IL-12 Induction

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Defective T cell receptor (TCR) signaling resulting in lower T cell function plays a crucial role in the pathogenesis of T cell immunodeficiency in leukemia. Previous studies have indicated that lower TCR ζ levels are a common characteristic of patients with leukemia, and upregulating TCR ζ could partially recover T cell function. In this study, we characterized the effect of the stimulating factor induction on the TCR ζ , *Zap-70*, and *Fc ϵ RI γ* levels, IFN- γ secretion, and the distribution and clonal expansion of TCR V β subfamilies in CD3⁺ T cells sorted from peripheral blood from acute myeloid leukemia (AML) patients. The induction included single stimulating factor or a combination with different cytokines (IL-2, IL-7, IL-2 + IL-7, IL-7 + IL-12, CD3, CD3 + CD28 antibody, CD3 + CD28 antibody + IL-2, and CD3 + CD28 antibody + IL-7) at 72 h. The results showed that increased TCR ζ and *Zap-70* levels with decreased *Fc ϵ RI γ* in T cells after induction, and different responses to cytokine in T cell from different cases may indicate the heterogeneity of T cells and different immune statuses in different AML cases. Increased IFN- γ levels in T cells from AML patients were detected after induction in the IL-12 + IL-7, CD3 + CD28 + IL-2, and CD3 + CD28 + IL-7 groups. Moreover, the number of TCR V β subfamily T cells expressed was increased; however, all of the TCR V β subfamily T cells in the AML patients could not be completely recovered after induction. In conclusion, the cytotoxicity and activation function of T cells could be enhanced after induction by different stimuli accompanied by an increase in TCR ζ and *Zap-70* and recovery of the TCR V β repertoire in AML patients.

Introduction

ACUTE MYELOID LEUKEMIA (AML) is the most common acute leukemia in adults; it is a progressive and malignant cancer of myelogenous blood cells and is associated with poor outcome. With intensive induction therapy, most patients younger than 60 years achieve complete remission. However, even if these younger patients are treated intensively, greater than 50% will relapse. Clinical results of patients older than 60 years are more unfavorable, and the overall survival for all AML patients remains low (Greiner *et al.*, 2008). Allogeneic stem cell transplantation is one alternative treatment for high-risk and relapsed/refractory AML. However, significantly associated nonrelapse mortality limits the application and outcome of this procedure. No currently available treatments have demonstrated consistent efficacy in terms of remission induction or long-term survival. Recently, targeted molecular therapies and immune-based therapies, such as the leukemia-associated antigen vaccine and antigen-specific cytotoxic T cells, are under

investigation for the management of high-risk AML (Greiner *et al.*, 2008; Sasine and Schiller, 2015). Long-term complete remission was demonstrated for some AML cases with cellular cytotoxic activity against myeloid leukemia cells (Müller-Schmah *et al.*, 2012).

T cell immunodeficiency plays an important role in cancer and leukemia progression and promotes the expansion of malignant clones (Greiner *et al.*, 2008; Müller-Schmah *et al.*, 2012). Recent studies have revealed that T cell immunodeficiencies are common characteristics of patients with AML, and they are characterized by peripheral T cells that are incapable of interacting with blasts, reduced thymic output function, oligoclonally restricted T cell repertoires, and low activation and response to antigens. Moreover, the immunosuppressive microenvironment in the bone marrow where both innate and adaptive immune responses are profoundly deregulated sustains and modulates the proliferation, survival, and drug resistance of AML (Li, 2008, 2014; Shi *et al.*, 2013; Isidori *et al.*, 2014). Cases in which low T cell activation may be due to abnormal T cell receptor (TCR)

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signal transduction, such as that involving the TCR ζ (also called as CD3 ζ) chain (Rezvan *et al.*, 1999; Chen *et al.*, 2000; Li *et al.*, 2009b; Erba, 2007), modulating the immune system, including reconstituting T cell activation and cytotoxicity against leukemia, may improve the survival of AML patients because the immune system is capable of recognizing and attacking leukemic cells (Isidori *et al.*, 2014).

TCR ζ is a CD3 subunit of the TCR/CD3 complex that plays a central role in T cell activation. Absence of the TCR ζ chain impairs the proliferative response and mature T cell activation level. T cells from patients with leukemia are functionally impaired, and this is related to a decrease in the TCR ζ chain expression (Chen *et al.*, 2000; Fischer *et al.*, 2005; Li, 2008; Chen *et al.*, 2011). Recently, we have reported significantly lower expression of TCR ζ in patients with AML and CML, and increasing TCR ζ expression by gene transfer enhances the activation and cytotoxicity of T cells from patients with CML (Chen *et al.*, 2009; Zha *et al.*, 2012a, 2012b). However, there are few data characterizing TCR ζ upregulation and the T cell activation and proliferation induced by different methods such as gene transfer and cytokine induction in T cells from AML. Thus, in this study, we investigated the characteristics of TCR ζ upregulation and the TCR V β polyclonal expansion repertoire in CD3 $^{+}$ T cells from patients with AML after IL-2, IL-7, and IL-12 induction.

Materials and Methods

Samples

The samples used in this study were obtained from four newly diagnosed, untreated (*de novo*) patients with AML, including two males and two females (17–48 years old; median age: 33.25) who were classified according to morphology based on criteria defined according to the FAB classification. The clinical data of the patients are listed in Table 1. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by Ficoll-Hypaque gradient centrifugation. CD3 $^{+}$ T cells were sorted from PBMCs using anti-CD3 monoclonal antibodies (MoAb) and the MACS magnetic cell sorting technique (Miltenyi Biotec). All procedures were conducted according to the guidelines of the Medical Ethics Committee of the Health Bureau of Guangdong Province, China.

Cell culture

CD3 $^{+}$ T cells from patients were suspended at $2\text{--}5 \times 10^5$ CD3 $^{+}$ T cells per mL per well in 24-well tissue culture plates in RPMI 1640 (Gibco) medium supplemented with 10%

heat-inactivated fetal calf serum and 100 U/mL penicillin/streptomycin. CD3 $^{+}$ T cells were stimulated with different combination cytokines (IL-2, IL-7, IL-2 + IL-7, IL-7 + IL-12, CD3 MoAb, CD3 + CD28 MoAb, CD3 + CD28 MoAb + IL-2, and CD3 + CD28 MoAb + IL-7) at 500 U/mL (PeproTech), 500 U/mL IL-7 (PeproTech), or 50 U/mL IL-12 (Sigma). The cultures were incubated in a humidified atmosphere at 37°C in 95% O $_2$ and 5% CO $_2$. After 3 days of culture, the cells were collected for RNA isolation, and analysis of the gene expression levels and TCR V β repertoire was performed.

RNA isolation and cDNA synthesis

RNA was extracted from the T cells before and after induction using the TRIzol RNA extraction buffer according to the manufacturer's protocol (TRIzol; Invitrogen). The quality of the RNA was analyzed in a 0.8% agarose gel stained with ethidium bromide. Two micrograms of RNA was reverse transcribed into first strand cDNA with random hexamer primers using the reverse transcriptase in the Superscript III Kit (Gibco). The cDNA quality was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) for $\beta 2$ microglobulin ($\beta 2M$) (RT-PCR primers for the $\beta 2M$ gene are listed in Table 2).

Real-time quantitative reverse transcription-polymerase chain reaction

The primers used in this study were purchased from Shanghai Invitrogen Biotechnology Co. Ltd. (Table 2). The expression level of the TCR ζ , *Zap-70*, and *Fc ϵ RI γ* genes in cDNA from stimulated or unstimulated CD3 $^{+}$ T cells was examined by SYBR Green I real-time PCR. Briefly, PCR was performed in a 20 μ L total volume containing 1 μ L cDNA, 9 μ L 2.5 $\times$ SYBR Green I master mix (Tiangen), and 10 μ M primers. After an initial denaturation at 95°C for 15 min, 45 cycles of the following procedure was performed using an MJ Research DNA Engine Opticon 2 PCR cycler (Bio-Rad): 30 s at 95°C followed by 40 s at 60°C for the $\beta 2M$, TCR ζ , *Zap-70*, and *Fc ϵ RI γ* genes. The relative amounts of the genes of interest and the $\beta 2M$ reference gene were measured in two independent assays. Specific amplification of PCR products was analyzed by melting curve analysis. The data are presented as the relative expression of the genes of interest compared with the internal control gene as determined by the $2^{(-\Delta CT)}$ method (Chen *et al.*, 2010; Shen *et al.*, 2013).

Western blot analysis

CD3 $^{+}$ T cells at $1\text{--}3 \times 10^5$ cells/0.2 mL with or without stimulation were solubilized in RIPA lysis buffer (1 $\times$ phosphate buffered saline [PBS], 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 10 mM phenylmethylsulfonyl fluoride, 1 mg/mL aprotinin, and 100 mM sodium orthovanadate) and incubated on ice for 30 min to isolate total protein. Proteins (10 μ g/well) were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (Pall) using a damp-dry transfer device (Bio-Rad). After blocking for 1 h in 5% nonfat milk in PBS, the membrane was washed and incubated with mouse anti- β -actin (1:2000; ProteinTech), rabbit anti-human TCR ζ or anti-human ZAP-70 (1:1000; Cell Signaling Technology).

TABLE 1. CLINICAL DATA OF ACUTE MYELOID LEUKEMIA PATIENTS

No.	Subtype	Sex	Age	WBC ($\times 10^9/L$)	Blast + promyelocyte cells (%)	Platelets ($\times 10^9/L$)
C1	M3	M	21	99.45	95	15
C2	M2	F	23	180.57	90	37
C3	M2	F	17	50.1	78	14
C4	M5	M	45	161.04	56	53

WBC, white blood cells.

TABLE 2. LIST OF PRIMERS USED FOR POLYMERASE CHAIN REACTION ANALYSIS

Primer	Sequence (5'-3')	GenBank ID	Product size (bp)
$\beta 2M$	5'-TACACTGAATTCACCCCCAC-3' 5'-CACTCAATCCAAATGCGGCA-3'	J00105	145
TCR- ζ	5'-GCCAGAACCAGCTCTATAAC-3' 5'-TAGGCCTCCGCC ATCTTATC-3'	NM198053.2	166
Zap-70	5'-GTTGACTCATCCTCAGAGACGAAT-3' 5'-AGGTTATCGTCGCGCTTCAGGAA-3'	NM001079.3	76
Fc ϵ RI γ	5'-GAGCCTCAGCTCTGCTATATCC-3' 5'-TCTCGTAAGTCTCCTGGTGCC-3'	NM004106.1	172

The antibodies were detected with 1:10,000 horseradish peroxidase-conjugated goat anti-rabbit IgG and horseradish peroxidase-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch). A western blotting Luminol reagent (Beyotime) was used to visualize bands corresponding to each antibody (Nambiar *et al.*, 2003). Blot images were captured using an Alliance 4.7 Gel doc system (UVI), and densitometric analysis of the bands was performed using the ImageJ software (<http://rsb.info.nih.gov/ij>).

RT-PCR for TCR V β subfamily gene analysis

Twenty-four TCR V β subfamily genes were amplified by RT-PCR using 24 TCR V β sense primers and a common TCR C β reverse primer. Subsequently, runoff PCR was performed with fluorescent primers labeled at the 5' end with a FAM fluorophore (C β -FAM) purchased from TIB MOL-BIOL GmbH. PCR was performed as previously described (Li *et al.*, 2005a). The 25 μ L reaction mixture contained 1 μ L cDNA, 0.5 mM forward and reverse primers, 0.1 mM dNTP, 1.5 mM MgCl₂, 1 $\times$ PCR buffer, and 1.25 U Taq polymerase (Promega). The amplifications were performed in a DNA thermal cycler (Biometra). After a 3 min denaturation at 94°C, 40 PCR cycles were performed with each cycle consisting of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final 6 min elongation at 72°C. The products were then stored at 4°C (Li *et al.*, 2005a; Jin *et al.*, 2014).

GeneScan analysis

Two microliters of FAM-labeled runoff PCR products were heat denatured with 9.5 mL Hi-Di formamide (ABI) and 0.5 mL size standards (GeneScanTM-500-LIZTM; ABI) at 94°C for 4 min. The samples were then loaded onto 3100 Performance Optimized Polymer-4 gel (3100 POP-4TM; ABI), resolved by electrophoresis in an ABI 3100 DNA sequencer and analyzed using the GeneScan software as previously described (Li *et al.*, 2005a; Jin *et al.*, 2014).

Enzyme-linked immunosorbent assay

Cell supernatants were collected from stimulated groups after 72 h, and the IFN- γ levels were measured using the human IFN- γ enzyme-linked immunosorbent assay (ELISA) kit (Raybiotech) according to the protocol of the manufacturer.

Statistical analysis

Differences in mRNA expression between two groups were analyzed using the paired Student's *t*-test. The data are presented as the mean $\pm$ standard deviation. Differences in

the IFN- γ levels were analyzed using nonparametric tests for K related samples. Differences with a *p* < 0.05 were considered statistically significant.

Results and Discussion

The expression levels of the TCR ζ , Zap-70, and Fc ϵ RI γ genes in AML T cells after cytokine induction

Because T cell immunodeficiencies are common characteristics of patients with AML (Li, 2008; Isidori *et al.*, 2014), we aimed to elucidate the characteristics of T cell immunity in patients with AML. Our previous studies have characterized reduced thymic output function, oligoclonally restricted T cell repertoires, and a low repression level for TCR signal transduction factors in T cells from AML patients (Li *et al.*, 2005a, 2009b; Chen *et al.*, 2011). We found significantly lower expression levels for all four of the chain genes, CD3 γ , δ , ϵ , and ζ , in CD3⁺ T cells, and the expression pattern of the four CD3 genes was different from that of healthy controls (Chen *et al.*, 2011). Such deficiency in CD3 gene expression levels might be, at least, a part of the reason for the reduction in T cell activation in AML. Similar findings were also reported in CML, CLL, and lymphomas (Huang *et al.*, 2012; Zha *et al.*, 2012b; Liao *et al.*, 2014). Therefore, enhancing CD3 expression may reactive the T cell activation functions and reverse the immunodeficiency in leukemia patients. Enhancement in TCR ζ (CD3 ζ), which is crucial for TCR signaling functions, because it provides 6 of 10 immunoreceptor tyrosine-based activation motifs for the TCR-CD3 complex (Call and Wucherpfennig, 2005; Li, 2008; Zha *et al.*, 2012a), was reported by different methods, including cytokine induction and TCR ζ transfer in T cells of CML patients (Chen *et al.*, 2009; Zha *et al.*, 2012b). A previous study showed that IL-2 could enhance the TCR ζ level, but it could not completely recover the TCR ζ level in T cells in CML patients (Chen *et al.*, 2009). In this study, we compared the effects of different cytokine combinations on enhancing the TCR ζ level.

We analyzed the effects of increasing the TCR ζ level in CD3⁺ T cells sorted from four cases with AML after induction with a single stimulating factor or a combination of different cytokines at 72 h *in vitro*. The TCR ζ expression level in CD3⁺ T cells from AML patients was mostly increased in each group (IL-2, IL-7, IL-2+IL-7, IL-7+IL-12, CD3 MoAb, CD3+CD28 MoAb, CD3+CD28 MoAb+IL-2, and CD3+CD28 MoAb+IL-7) (Fig. 1) with the exception of the IL-2+IL-7 and CD3+CD28 MoAb+IL-2 combinations in Case 2 (M2), and the TCR ζ expression level in CD3⁺ T cells after induction appeared to be slightly decreased. Interestingly, there was an increased TCR ζ expression level in all

FIG. 1. The expression level of the *TCRζ* and *Zap-70* genes in T cells from four cases with acute myeloid leukemia (AML) before or 3 days after induction. A: Case 1 (AML-M3), B: Case 2 (AML-M2), C: Case 3 (AML-M2), D: Case 4 (AML-M5b).

groups in Case 3 (M2). The different response to cytokine induction for the T cells in the M2 cases may indicate T cell heterogeneity and different immune statuses in different cases even within the same AML subtype.

We next detected the *Zap-70* expression level in the same samples used for *TCRζ* detection. An increased *Zap-70* expression level could be detected in each group in Case 4 (M5), whereas slight up or downexpression was found in Cases 1 through 3 in different groups in which the same tendency for *Zap-70* upexpression was found in the CD3+CD28 MoAb and CD3+CD28 MoAb+IL-7 groups (Fig. 1). The expression levels of the *TCRζ* and *Zap-70* proteins were

also measured by western blot analysis, and similar results were found. An increased *TCRζ* level was found in the IL-2+IL-7 ($p=0.01$) and CD3+CD28 MoAb+IL-7 groups, and increased *Zap-70* was found in the IL-12+IL-7 group ($p=0.02$) (Fig. 2). These results suggested that *TCRζ* and its downstream factor *Zap-70* could be enhanced by the combination of IL-2 IL-7 or IL-12, and IL-7 may be the most important factor for such a stimulation process. It is well known that IL-7 is required for the development and survival of T cells and plays a critical role in modulating T cell homeostasis. This cytokine costimulates T cell activation by enhancing proliferation and cytokine production, particularly

FIG. 2. Detection of *TCRζ* and *Zap-70* protein in T cells from an AML case before and after induction in different groups.

FIG. 3. The expression level of the *FcεRIγ* gene in T cells from four cases with AML before or 3 days after induction. A: AML-M3, B: AML-M2, C: AML-M2, D: AML-M5b.

in the setting of suboptimal TCR triggering. Several studies have shown that IL-7 enhances T cell survival in long-term cell cultures, and it was shown to be superior to IL-2. Moreover, IL-12 as a cytotoxic lymphocyte maturation factor/NK cell stimulatory factor, can enhance IL-2 production and the activity of cytotoxic effector cells, the combination of IL-7 and IL-12 is synergistic, leading to IFN- γ responses greater than that of IL-12 alone (Soiffer *et al.*, 1993; Lynch and Miller, 1994; Fry and Mackall, 2002; Plain *et al.*, 2012). Such a phenomenon was also shown in this study.

We also analyzed the expression characteristics of *FcεRIγ*, the TCR ζ gene complementary factor. The expression level of the *FcεRIγ* gene was downregulated in each group and all samples after induction (Fig. 3). The expression level of *FcεRIγ* was highest in Case 4, which was as high as 27- and 69-fold greater than Cases 1 and 2, respectively, followed by Case 3 before induction. This result was consistent with the results for the TCR ζ expression level, which was lowest in Case 4 followed by Case 3 before induction, and this may be because both expression levels are complementary (Niu *et al.*, 2015). This phenomenon also indicated a heterogeneous immune status for the AML patients. It is reasonable that the *FcεRIγ* expression level was downregulated after induction because, in this case, TCR ζ expression was upregulated.

Increased IFN- γ secretion in T cells from AML after cytokine induction

We next examined T cell function by measuring the IFN- γ secretion level (Thomas *et al.*, 2014), which was stimulated by a single stimulating factor or a combination of different cytokines at 72 h *in vitro*. The secretion of IFN- γ from T cells from AML patients was significantly increased in each group ($p=0.039$). An increased IFN- γ level was detected in the IL-12+IL-7, CD3+CD28 MoAb+IL-2, and CD3+CD28 MoAb+IL-7 groups (Fig. 4). Thus, these results suggested that the cytotoxicity of T cells was enhanced after induction by different stimuli and was accompanied by an increase in TCR ζ and Zap-70 and T cell activation, confirming that T cell function could be enhanced by cytokine induction in AML patients.

The distribution and clonality of CD3⁺ T cells from AML patients after cytokine induction

Different TCR subfamily T cells display different functions for specific antigen responses (Zha *et al.*, 2011; Chen *et al.*, 2013), thus, it is of interest to characterize the contribution of cytokine induction to TCR subfamily T cell clone proliferation. Using results from the expression characteristics of the TCR ζ , Zap-70, and *FcεRIγ* genes and the IFN- γ secretion of CD3⁺ T cells from AML patients after cytokine

FIG. 4. The IFN- γ secretion level in T cells from AML patients after cytokine induction.

induction, we analyzed changes in the TCR V β repertoire and T cell clonality in CD3⁺ T cells from three groups (control, CD3+CD28 MoAb, and CD3+CD28 MoAb+IL-7).

In general, 24 of the TCR V β subfamilies could be detected in peripheral blood T cells from healthy individuals (Li *et al.*, 2007, 2009a), however, the absence of some TCR subfamilies is frequent in leukemia samples, which is a common characteristic of leukemia patients related to T cell immunodeficiency (Li *et al.*, 2005a; Jin *et al.*, 2014). In this study, 11–20 TCR V β

subfamilies could be detected in CD3⁺ T cells from four cases with AML, and most cases displayed polyclonal expansion. Four to 13 TCR V β subfamilies were absent in the different cases, and previous studies showed a skewed distribution for the TCR V β repertoire, which was mostly detected in PBMCs from AML patients (Li *et al.*, 2005a). These results may be influenced by the fact that PBMCs contain a high percentage of AML cells, which may reduce the sensitivity of PCR analyses. In this study, we detected the expression profile of the TCR V β

FIG. 5. T cell clonality in CD3⁺ T cells from AML patients after cytokine induction. C1 to C4: AML Cases 1 to 4, A: control, B: CD3+CD28 monoclonal antibodies (MoAb) induction, C: CD3+CD28 MoAb+IL-7 induction.

repertoire in purified CD3⁺ T cells from AML patients, and the results may directly reflect the distribution of the TCR V β repertoire. Similar findings have indicated T cell immunodeficiency with restricted expression of the TCR V β repertoire in AML patients. Not only the low activation, but also the abnormal expansion of the TCR V β subfamily T cell needs to be revived in AML patients for T cell immunotherapy. Therefore, in this study, we attempted to revive T cell activation and expansion by using different T cell stimuli after confirming that the expression levels of the TCR ζ and *Zap-70* genes and the IFN- γ secretion ability of CD3⁺ T cells from AML patients was increased in different stimulation groups. In addition, we also analyzed the expression and expansion of the TCR V β subfamilies. Overall, the number of expressed TCR V β subfamilies was increased in T cells from the same case after induction; however, unlike the induction in normal T cells, which showed complete expansion of TCR V β subfamily T cells after IL-2 or PHA induction (Li *et al.*, 2005b), stimulation could not completely recover all of the TCR V β subfamily members. For example, V β 8 was not detected in any of the samples, whereas V β 24 was positive after CD3+CD8 MoAb+IL-7 induction in Cases 3 and 4, but not in Case 1. This result may be related to the abnormal T cell function in AML patients, which could not be completely reversed by cytokine induction. However, another possibility is that there are specific clonally expanded T cells, which were thought to be related to leukemia-associated antigen (Li *et al.*, 2005; Jin *et al.*, 2014) in AML patient samples. Therefore, we further analyzed the clonality of the T cells before and after induction. Most T cells displayed polyclonality before induction, and only V β 19 (in Case 2), V β 6, V β 15, and V β 18 (Case 3) and V β 9 (Case 4) showed oligoclonality. In addition, most TCR V β subfamily members maintained the same clonality after induction, for example, oligoclonally expanded V β 9 T cells were identified in Case 4 before induction, and similar clonal characteristics were found for T cells after CD3+CD28 and CD3+CD28+IL-7 stimulation. These data suggest that a specific antigen for the clonally expanded T cells may be the leukemic-associated antigen. While some oligoclonally expanded T cells could be altered to have polyclonal proliferation, for example, oligoclonally expanded V β 6, V β 15, and V β 18 T cells were identified in Case 3 before induction and the clonality was changed to polyclonality after CD3+CD28 MoAb and CD3+CD28 MoAb+IL-7 induction, it is interesting to note that polyclonality changing to oligoclonality was also observed for some V β subfamily members after induction, for example, V β 20 in Case 3 and V β 15 in Case 4 (Fig. 5). It is thought that the change in T cell clonality from oligo- to polyclonality indicates recovery of the proliferation of T cells after cytokine induction, but it is difficult to explain the change in T cell clonality from poly- to oligoclonality, which remains an open question requiring further investigation in a larger cohort of samples.

In conclusion, we characterized alterations in the TCR ζ , *ZAP-70*, and *Fc ϵ RI γ* expression levels and increased IFN- γ secretion in AML T cells after induction by different cytokine combinations, and IL-7 might play a critical role in TCR ζ upregulation and T cell activation. Moreover, the number of expressed TCR V β subfamily T cells was increased; however, all TCR V β subfamily T cells could not be completely recovered in AML patients after induction. Overall, the cytotoxicity and activation function of T cells could be enhanced after different stimuli, which accompanied a TCR ζ

and Zap-70 increase and recovery of the TCR V β repertoire in AML patients. Further investigation is needed to determine the optimal combination of cytokines for enhancing T cell expansion. Moreover, such T cell immune activation might be considered for supporting to design the TCR or CAR modified T cell immunotherapy in future (Han *et al.*, 2013).

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Authors' Contributions

S.H.C. and Y.Q.L. contributed to the concept development and study design. S.L., X.F.Z., Y.X. and L.X. performed real-time PCR, RT-PCR, GeneScan, cell culture, ELISA, and western blot assays. S.L. and L.J.Y. prepared PBMCs, RNA, and cDNA. Y.H.L. and K.E.Z. were responsible for clinical diagnoses and performed clinical data acquisition. Y.Q.L., S.H.C., and S.L. coordinated the study and helped draft the article. All authors read and approved the final article.

Disclosure Statement

No competing financial interests exist.

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