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Changes of Th17 / IL-17A in Patients with Acute Myeloid Leukemia Before and After Treatment and Its Effect on Prognosis

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Abstract

Objective: To study the changes of Th17 cell percentage and IL-17A level in peripheral blood of patients with acute myeloid leukemia (AML) before and after treatment, analyze the role of Th17 / IL-17A in the pathogenesis of AML, and analyze the changes of Th17 / IL-17A in different stages of AML after treatment and its impact on prognosis. Methods: from September 2017 to June 2020, 38 patients with newly diagnosed AML in our hospital were selected as the study group, and 28 patients with iron deficiency anemia were selected as the control group. The levels of Th17 cells and IL-17A in peripheral blood of patients with AML and iron deficiency anemia were detected by flow cytometry. Objective to analyze the changes of Th17 / IL-17A before treatment and 0-3 months, 3-6 months after treatment, and analyze the relationship between Th17 / IL-17A and the prognosis of AML. Results: the proportion of Th17 (CD3 + CD4 + IL-17 +) cells in the peripheral blood of newly diagnosed AML patients was (2.74 ± 0.85)%, which was significantly higher than that of the control group (1.02 ± 2.12)%, the difference was statistically significant (P < 0.05). The IL-17A level in the peripheral blood of AML patients before treatment was (3.16 ± 1.54) pg / ml, which was significantly higher than that of the control group (2.22 ± 0.21) pg / ml, the difference was statistically significant. The levels of Th17 cells and IL-17A in peripheral blood of patients with complete remission after initial induction therapy were (3.31 ± 0.31%; 6.32 ± 0.73 pg / ml) higher than those before induction therapy, the difference was statistically significant. The levels of Th17 cells and IL-17A in peripheral blood of AML patients from 0 to 3 months after complete remission were (1.94 ± 0.11%; 4.28 ± 0.51pg / ml) lower than those in complete remission, the difference was statistically significant; the levels of Th17 cells and IL-17A in peripheral blood of AML patients from 3 to 6 months after complete remission were (1.26 ± 1.58%; 2.46 ± 0.32 pg / ml) lower than those in complete remission, the difference was statistically significant (P < 0.05). Compared with the control group, the levels of Th17 cells and IL-17A in peripheral blood were slightly higher 3-6 months after complete remission (P > 0.05). Conclusion: the levels of Th17 cells and IL-17A in peripheral blood of patients with AML are significantly increased. Th17 cells and IL-17A play an important role in the immune pathogenesis of AML. The immune mechanism of AML patients gradually returned to normal 3-6 months after complete remission.

Keywords

AML; Th17 / IL-17A; Flow cytometry.

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1. INTRODUCTION

Acute myeloid leukemia (AML) is the most common malignant clonal disease of hematopoietic system, mainly characterized by the increase of immature myeloid cells in bone marrow. Chemotherapy is the main method for the treatment of AML. At present, with the improvement of AML treatment, the remission rate has been greatly improved, but the recurrence and drug resistance seriously affect the prognosis, so it is urgent to find a new treatment method. In recent years, the immunotherapy of AML has attracted more and more attention, and achieved certain results. At present, studies have found that the occurrence and development of AML is closely related to the disorder of immune environment, especially the immune abnormality mediated by cellular immunity. T helper cells (Th cells) play an important role in cellular immunity, which can be divided into Th1, Th2, Th17 and regulatory T cells (Treg). In the past, there were many studies on Th1 and Th2. Th17 cells are new helper T cells discovered in recent years. They are a group of cell subsets induced by TGF - β and IL-6 [1], and play an important role in autoimmune diseases and body defense response [2]. IL-17A is the main specific cytokine secreted by Th17 cells. In recent years, the immune mechanism of Th17 cells and IL-17A in tumor microenvironment has become the focus of immunotherapy. Previous studies have shown that Th17 cells and IL-17A play different roles in different tissue types of tumors, and the specific mechanism is unknown, which needs further study. At present, there are few studies on the role of Th17 and IL-17A in the pathogenesis and prognosis of AML. Therefore, this experiment detected the levels of Th17 and IL-17A in peripheral blood of newly diagnosed AML patients, and studied the relationship between Th17 and IL-17A and clinical characteristics of AML patients, and explored their role and mechanism in the occurrence and development of the disease.

2. DATA AND METHODS

2.1. Clinical Data

The 38 patients were all newly diagnosed acute myeloid leukemia patients in the Department of Hematology of Binzhou people's Hospital from September 2017 to June 2020. According to the who MICM diagnostic criteria, they were diagnosed by bone marrow cell morphology, immunology, cytogenetics and molecular biology, and the risk was stratified. There were 16 cases in the low-risk group, 18 cases in the medium risk group, 4 cases in the high-risk group, 22 males and 16 females The median age was 42 (13-72) years. The control group consisted of 28 patients with iron deficiency anemia, 18 males and 10 females, with a median age of 36 (29-52) years. This study was approved by the ethics committee of Binzhou people's Hospital, and informed consent was signed with patients.

2.2. Method

2.2.1 in the morning, 2ml of whole blood anticoagulated with fasting heparin was collected and left at room temperature for 30 minutes.

2.2.2 stimulation: take 50 μ l anticoagulant whole blood into durative1 tube, vortex for 6-8 seconds, cover the tube cover, and incubate in carbon dioxide incubator at 37°C for 3 hours.

2.2.3 flow cytometry was used to detect Th17 cells

2.2.3.1. Add 25 μ l buffer R1 solution (perfix NC fixative reagent) to durative 1 tube after stimulation. After vortex, incubate at room temperature for 15 minutes.

2.2.3.2. Add 2 ml of 1 x PBS (calf serum), vortex and centrifuge at 200 x g (1700 RPM) for 5 minutes, suck up the supernatant, add 25 μ l fetal bovine serum, vortex.

2.2.3.3. Add 300 μ l buffer R2 (perfix NC) and swirl

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2.2.3.4. Transfer all liquid in durative1 tube to duraclone if T helper cell panel tube. High speed vortex centrifuge tube for 6-8 seconds. Incubate at room temperature for 45 minutes (antigen reactive antibody). Dilute R3 solution 10 times (with distilled water, about 4ml for each sample).

2.2.3.5. Add 3 ml R3 (final solution 1x in water) (perfix NC Water Reagent) to each tube, vortex and centrifuge 500 g for 5 minutes, discard the supernatant, add 500 μ l R3 (final solution 1x in water), vortex suspension, and test on the machine (in th scheme). The ratio of Th17 cells to CD4 + T cells in peripheral blood was used to evaluate the expression level of Th17 cells.

2.2.4 detection of serum IL-17A concentration

2.3. Statistical Methods

SPSS 21.0 statistical software was used for data analysis. The measurement data was expressed as mean ± standard deviation ($\bar{x}\pm s$). If the measurement data obeyed normal distribution, t test was used for comparison between the two groups. If not, Wilcoxon rank sum test was used for comparison between the two groups. $P < 0.05$ was statistically significant.

3. RESULTS

3.1. Comparison of the Proportion of Th17 Cells in Peripheral Blood Between the Two Groups

Flow cytometry showed that the proportion of Th17 cells in the peripheral blood of newly diagnosed AML patients was (2.74 ± 0.85%), and that of the control group was (1.02 ± 2.12) %, the difference was statistically significant ($P < 0.05$); see Figure 1, Figure 2 and Figure 3.

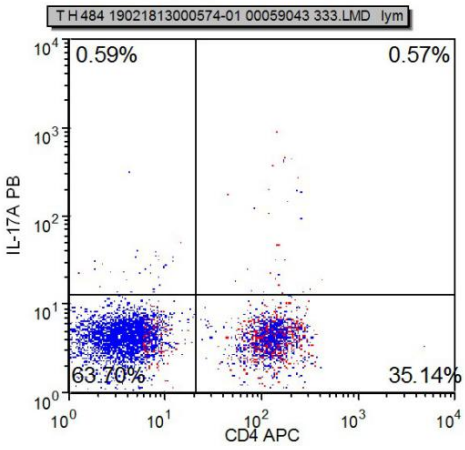

Figure 1. Expression level of Th17 in peripheral blood of control group (%)

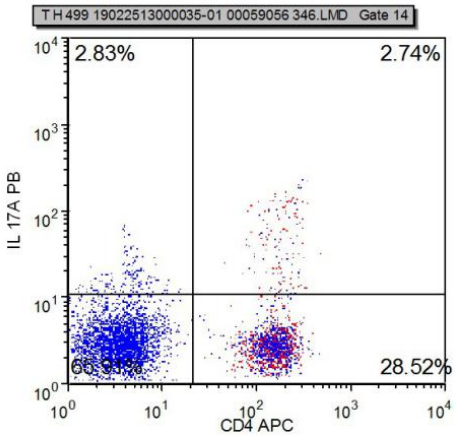

Figure 2. Expression of Th17 in peripheral blood of AML patients (%)

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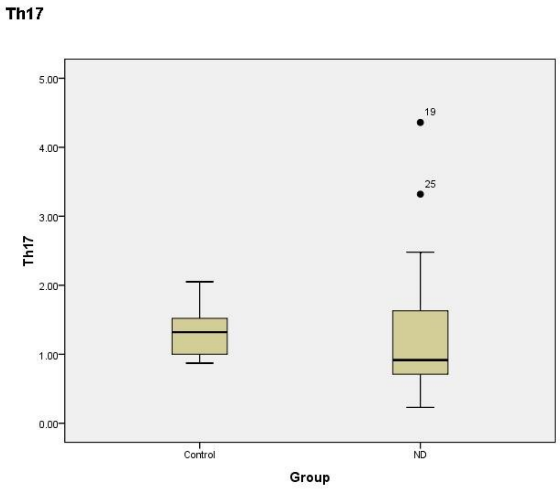

Figure 3. Percentage of Th17 in AML and control group

3.2. Comparison of IL-17A Levels in Peripheral Blood Between the Two Groups

The results of flow cytometry showed that the level of serum IL-17A in the study group was (3.16 ± 1.54) pg / ml, which was significantly higher than (2.22 ± 0.21) pg / ml in the control group ($P < 0.05$), as shown in Figure 4.

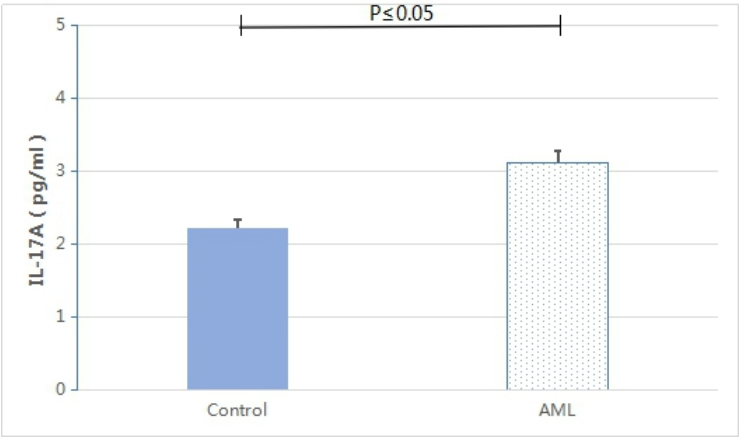

Figure 4. Expression of IL-17A in AML and control group

3.3. The Changes of Th17 and IL-17A Levels in Peripheral Blood at Different Stages after Complete Remission

Among 38 newly diagnosed AML patients (except m3), 4 of them gave up regular chemotherapy due to economic reasons and only received blood transfusion support treatment. 34 patients were treated with standard induction therapy. Among them, 24 patients achieved complete remission after one course of treatment, 6 patients achieved complete remission after two courses of treatment, and 4 patients gave up continuing treatment because they did not achieve complete remission after two courses of chemotherapy. In 30 patients with complete remission after induction therapy, the percentage of Th17 in peripheral blood at CR was 3.31 ± 0.31%, and the level of IL-17A was 6.32 ± 0.73 pg / ml, which were higher than the levels of Th17 and IL-17A at initial diagnosis (2.74 ± 0.85%, 3.16 ± 1.54 pg / ml) (see Figure 5). The levels of Th17 and IL-17A in peripheral blood at 0-3 months after Cr were 1.94 ± 0.11% and 4.28 ± 0.51 pg / ml respectively, which were significantly lower than those at 0-3 months after Cr ($P < 0.05$))Compared with the control group, the difference was not statistically significant ($P > 0.05$).

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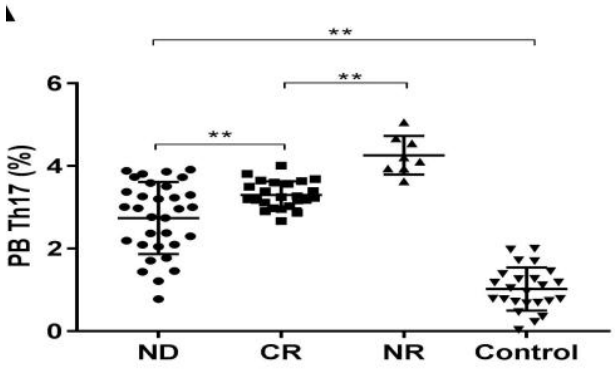

Figure 5. Percentage of Th17 cell subsets in peripheral blood of newly diagnosed AML patients and Cr and NR patients after induction therapy

Table 1. Expression of Th17 and IL-17A in different intervals after AML treatment

	control group	CR	0-3month	3-6month
Th17(%)	1.02±2.12%	3.31±0.31%	1.94 ±0.11%	1.26±1.58%*
IL-17A(pg/ml)	2.46 ± 0.32	6.32 ± 0.73	4.28±0.51	2.46±0.32*

*Compared with the control group, P > 0.05, the difference was not statistically significant

4. DISCUSSION

Acute myeloid leukemia (AML) is a malignant clonal disease originated from hematopoietic stem cells. Its pathogenesis is still unclear. Recent studies have shown that its occurrence and development are closely related to the abnormal immune level of the body, especially the imbalance of Th cell subsets.

Helper T cells (Th cells) play an important role in cellular immune regulation. The disorder of Th cell subsets is closely related to the disorder of immune microenvironment. The disorder of immune microenvironment is closely related to a variety of autoimmune diseases and tumors. Under different microenvironments, initial CD4 + T cells can differentiate into different T cell subsets, mainly including Th1, Th2, Th17 and Treg cells. These cells participate in the immune response of the body mainly by releasing different cytokines. In recent years, the immune mechanism of Th17 cells and IL-17A in tumor microenvironment has become a research hotspot.

Th17 cells are a class of helper T cell subsets that can secrete characteristic IL-17A, which are characterized by CD45 + CD4 + IL-17A +, and express specific transcription factor orphan receptor (orphan nuclear) Receptor (ROR γ T) has a strong pro-inflammatory effect, participates in the occurrence and development of many inflammatory diseases, autoimmune diseases and tumors [3], and also plays an important role in normal hematopoietic regulation. Th17 cells mainly secrete IL-17A, IL-17F, IL-21, IL-22, GM-CSF, IFN - γ and other inflammatory factors to exert immune and inflammatory effects [4]. IL-17A is usually referred to as IL-17, which has high homology with IL-17F, so its biological characteristics are similar. At present, there are still many controversies about the role of Th17 cells in the occurrence and development of tumors. In different types of tumors, Th17 cells may play an anti-tumor or anti-tumor effect by secreting different cytokines.

IL-17A is the most widely studied cytokine in the IL-17 family. It is also the main effector molecule secreted by Th17 cells. It is also referred to as IL-17 for short. It can activate mitogen activated protein kinase (MAPK) and NF - κ B pathway by binding to the cell surface receptor il-17ra, Regulating the expression of a variety of downstream target genes, and then promoting a

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variety of inflammatory cells or stromal cells to secrete a variety of inflammatory factors, play a key role in the occurrence and development of a variety of inflammatory related diseases and tumors. In tumor microenvironment, IL-17A induces tumor cells and immunosuppressive cells to secrete various cytokines and chemokines, causing inflammatory reaction, promoting tumor growth and angiogenesis [6]. Research [7] has proved that angiogenesis plays an important role in IL-17A promoting tumor proliferation, and cytokines can induce angiogenesis through direct or indirect mechanisms. Although most studies [8-10] show that IL-17A can promote cancer, some studies [11] show that IL-17A can inhibit tumor. The role of IL-17A in tumor microenvironment is different, which may be related to tumor type, etiology, development and complex tumor microenvironment. In recent years, more and more attention has been paid to the role of Th17 cells in the pathogenesis of acute leukemia. However, whether Th17 cells are involved in the pathogenesis of Al and whether they can be used as indicators to evaluate the efficacy and prognosis of Al are still controversial.

In this study, we observed that the level of Th17 cells in the peripheral blood of patients with AML was significantly higher than that of normal people, suggesting that Th17 cells participate in the immune response of patients with AML, which may mediate the anti-tumor immunosuppression and immune escape of tumor cells in patients with AML, play an important role in regulating the immune function of AML, and participate in the pathogenesis of AML. IL-17A is a specific factor secreted by Th17 cells. Studies have found that the expression of IL-17A in serum of AML group is higher than that of control group, suggesting that Th17 may participate in the pathogenesis of AML by secreting corresponding functional factors. In this study, we also observed the levels of Th17 and IL-17A in the peripheral blood of AML patients at CR after induction therapy and 0-3 months, 3-6 months after cr. the results showed that the levels of Th17 and IL-17A in the peripheral blood of AML patients at CR after induction therapy were higher than those before treatment. There was no significant difference between the levels of Th17 and IL-17A in the peripheral blood of AML patients at 3-6 months after Cr and the control group The function is close to normal level.

In conclusion, this study analyzed the expression of Th17 and IL-17A in peripheral blood of newly diagnosed patients with acute myeloid leukemia. The results showed that patients with acute myeloid leukemia had immune dysfunction, and Th17 and IL-17A may provide new targets for immunotherapy of AML. Due to the influence of chemotherapy drugs and disease itself, the immune function of AML patients did not recover until 3-6 after CR. At present, the number of research cases is limited, and large sample clinical studies are still needed to provide evidence.

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