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ORIGINAL ARTICLE

Analysis of Peripheral Blood T Lymphocyte Subsets in Multiple Myeloma Patients

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SUMMARY

Background: **The aim of this study was to investigate the changes of T lymphocyte subsets (Th1, Th2, Tc1, Tc2, and Th17) and memory T lymphocyte subsets (Tcm and Tem) in patients with multiple myeloma (MM) at different stages of the disease.**
Methods: **In total, 25 newly diagnosed patients with MM were selected as the study subjects and 30 healthy people were selected as the control group. The subsets of T lymphocytes such as Th1, Th2, Tc1, Tc2, Th17, Tcm, and Tem in the peripheral blood were detected by flow cytometry at the time of initial diagnosis, infection, and remission.**
Results: **Th1, Tem, and Tcm cells in MM patients showed a significant decrease compared to the control group. Th2 and Th17 cells in MM patients showed a significant increase compared to the control group. Total Th1 cells and memory Th1 cells in MM patients with bacterial infection were significantly higher than at initial diagnosis ($p < 0.05$). The Tcm of Th2 cells in the remission stage were significantly higher than those in MM patients with no remission.**
Conclusions: **MM patients have decreased Th1 cells and increased Th2 and Th17 cells. The changes in memory Th1 cells were related to bacterial infection in MM patients. The increase of Tcm of Th2 cells may be associated with disease remission. The balance of T lymphocyte subsets plays an important role in the pathogenic course of MM.**
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KEYWORDS

multiple myeloma, T lymphocytes, Th1, Th2, Tem, Tcm

INTRODUCTION

Multiple myeloma (MM) is a plasma cell neoplasm that accounts for 1% - 1.8% of all cancers, it is the second most common hematological malignancy and is correlated with increased immunoglobulins [1]. Given the abnormal proliferation of malignant plasma cells during immune escape, monoclonal immunoglobulins of undetermined significance result in abnormalities in cellular immunity and humoral immunity. Therefore, T lymphocytes involved in immune responses play an important

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role in MM immunosuppression and immune escape. T lymphocytes mainly include CD4+ T cells and CD8+ T cells, which play important roles in organisms. CD4+ T cells can be classified into other subsets, such as T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, T-helper 17 (Th17) cells, and CD4+CD25+ T regulatory (Treg) cells [2]. CD8+ T cells can be classified into T-cytotoxic type 1 lymphocytes (Tc1) and T-cytotoxic type 2 lymphocytes (Tc2) cells. Furthermore, memory T lymphocyte subsets were classified into central memory T cells (Tcm) and effector memory T cells (Tem) based on the expression of lymphoid-homing molecules CCR7 and CD62L (L-selectin) [3].

In our study, to explore whether T lymphocytes play a vital role in the development of MM, we investigated the proportions of Th subsets, Tc subsets, and memory T lymphocyte subsets. Moreover, changes of associated T cells were analyzed at different stages of the disease. This study aimed to provide a stronger basis for immune mechanisms, clinical treatment targets, and MM-related prognosis assessment.

MATERIALS AND METHODS

Subjects and materials

From January 2022 to March 2023, 25 newly diagnosed MM patients (according to International Myeloma Working Group criteria) were admitted to the hospital. The MM group included 16 males and 9 females, with a median age of 56 years. As control group, 30 people were selected, including 20 males and 10 females, with a median age of 59 years.

All MM patients received a combination chemotherapy based on bortezomib and immunomodulators and accepted maintenance therapy after 4 cycles of chemotherapy. The efficacy was evaluated after 4 cycles of chemotherapy [4]. Then according to the efficacy, 25 patients were divided into remission group and non-remission group. The peripheral blood was collected in EDTA anticoagulant tubes at the time of initial diagnosis, after 4 cycles of treatment, and at the time of infection.

The T lymphocyte subsets of the peripheral blood, such as Th1 (CD3+CD4+CXCR3+CD45RO+CCR6-), Th2 (CD3+CD4+CXCR3-CD45RO+CCR6-), Th17 (CD3+CD4+CXCR3-CD45RO+CCR6+), Tc1 (CD3+CD8+CXCR3+CD45RO+CCR6-), Tc2 (CD3+CD8+CXCR3-CD45RO+CCR6-), Tcm (CD45RO+CCR7+), and Tem (CD45RO+CCR7-), were detected on flow cytometry (BECKMAN COULTER NAVIOS). All the reagents were purchased from the American BD Company.

Statistical analysis

Data were analyzed by using SPSS Statistics 25.0 software (IBM Corp., Armonk, NY, USA). Groups were compared by using the independent sample *t*-test. Data were reported as means ± SE. A p-value < 0.05 denoted a statistically significant difference.

RESULTS

Th1, Th2, Th17, Tc1, Tc2 cells, and memory cells of Th1 and Th2 in MM patients and in the control group

Th2% and Th17% were significantly higher in MM patients than in the control group. Th1% was significantly lower in MM patients than in the control group ($p < 0.05$). Tcm% and Tem% of Th1 lymphocytes in MM patients significantly decreased, compared to the control group ($p < 0.05$). In contrast, there was no difference in other cells between patients and controls ($p > 0.05$) (Figure 1).

Changes of T cell subsets in MM patients with bacterial infection

Bacterial infections occurred in 13 MM patients during chemotherapy. The levels of Th1%, Tcm% of Th1, Tem% of Th1 in bacterial infected MM patients were significantly higher than at initial diagnosis. The differences were statistically significant ($p < 0.05$) (Figure 2).

Changes of T cell subsets in different clinical stages of MM patients

In the remission stage, Tcm% of Th2 in MM patients was higher than in MM patients with no remission ($p < 0.05$). In contrast, there was no difference in other cells between patients and controls ($p > 0.05$) (Figure 3).

DISCUSSION

MM is an incurable cancer characterized by the development of malignant plasma cells. Recent studies have shown that imbalance in T-cell subsets plays an important role in MM. With the genomic changes that occur in plasma cells, the bone marrow (BM) microenvironment enables immune escape and supports the progression of MM and the development of drug resistance [5]. T lymphocytes are important components of the BM microenvironment. Several functional and numerical defects in T lymphocytes have been identified in MM. Notably, CD4+ T cells are associated with disease progression and poor prognosis [6]. Effector CD4+ T cells can be divided into Th1, Th2, Th17, and Treg subsets, according to their differentiation and function [2]. Th1 and Th2 cells function in cellular and humoral immunity, respectively, while Th17 cells are involved in innate immunity [7]. In our study, Th1 cells were lower, while the Th2% and Th17% were significantly higher in MM patients than in healthy people. As a report concluded [8], Th1 cells could secrete a large amount of interferon-γ (IFN-γ) and display significant suppressive effects on the growth and function of myeloma cells. However, Th2 cells, which produce mainly interleukin-4 (IL-4), could suppress Th1 cell activation and contribute to humoral immunity. Furthermore, Th2 cells may even promote MM progression and enhance cytokine secretion of myeloma cells [8]. Th17 cells, which secrete of inter-

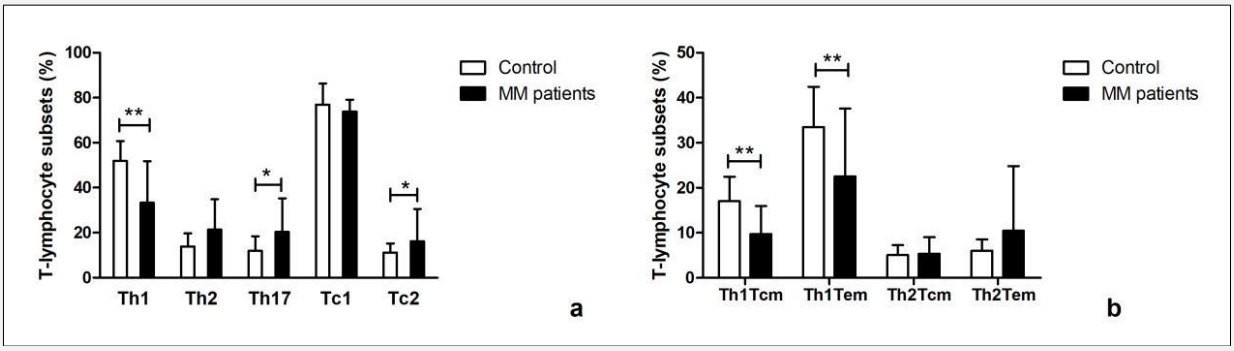

Figure 1. Comparison of T-lymphocyte subsets between newly diagnosed MM patients and controls (* p-value < 0.05, ** p-value < 0.01).

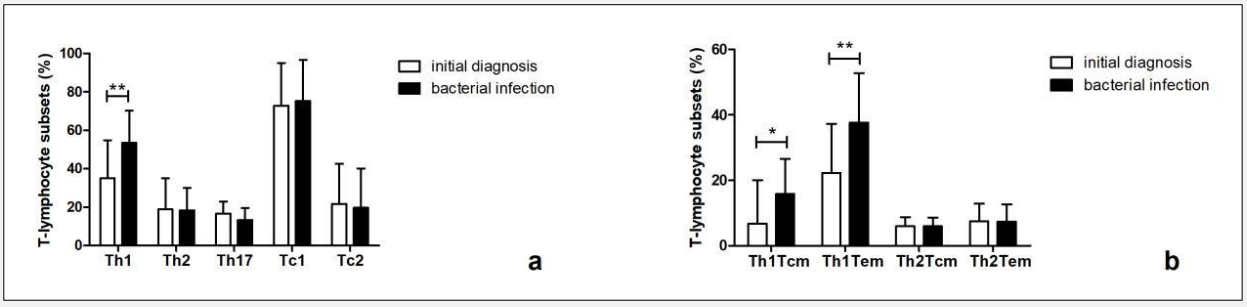

Figure 2. Comparison of T-lymphocyte subsets between patients with bacterial infection and the corresponding patients at initial diagnosis (* p-value < 0.05, ** p-value < 0.01).

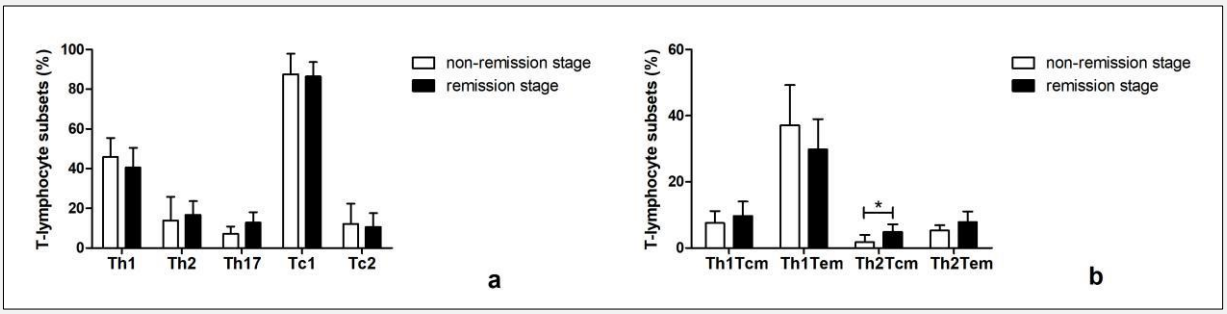

Figure 3. Comparison of T-lymphocyte subsets of MM patients in remission and non-remission stages (* p-value < 0.05).

leukin-17 (IL-17), were also found to play vital roles in MM-related bone disease. Meanwhile, IL-17 was recently validated as a growth factor for MM and a potent inducer of osteoclast activity [9]. IL-17 can also stimulate the expression of receptor activators of NF-κB in osteoblasts, which in turn promotes bone destruction [10]. Therefore, the imbalance of Th1, Th2, and Th17 cells were likely to be a key factor involved in the pathogenesis of MM.

Upon antigen stimulation, naive T cells enter distinct cell programs for development and differentiation and then differentiate to memory cells, which include Tscm, Tcm, and Tem [11]. Memory T cells play an important antitumor role. Tcm cells have a stronger antitumor effect than Tem cells, because they express more lymph node-homing mediators [12]. However, the expression of Tcm and Tem cells in MM patients at different stages of the disease is still unclear. In this study, we explored the percentages of Tcm and Tem cells in newly diagnosed MM patients and in different phases of MM patients. We found that Tcm% and Tem% of Th1 in MM patients were significantly lower than those in healthy people. These findings suggest that the decrease in Th1 memory cells is involved in the process of MM. Most importantly, in the absence of Th1 memory cells, the ability of memory CD8+ T cells to efficiently control a secondary pathogen or tumor challenge are also impaired [13-15].

Memory CD4+ T cells play immune responses against pathogens via the antigen-induced secretion of potent effector cytokines. The memory cells survive for months after immunization with antigen; one, which are found primarily in the lymph nodes, mainly produce IL-2, and the other, which are found in nonlymphoid tissues, mainly produce IFN-γ [16]. Tem was generally found to be associated with chronic infection [17]. One study suggested that while chronic *P. chabaudi* infection predominantly generates long-term Tem cells, the dominance of Tem cells over Tcm cells is actually determined within 3 to 5 days post infection [18]. In our study, when MM patients had a bacterial infection, the Tcm% and Tem% of Th1 cells significantly increased. Furthermore, the Tem of Th1 cells showed a more pronounced increase than the Tcm. These findings showed that Tem of Th1 cells were associated with bacterial infection in MM patients.

In addition, we found that the Tcm% of Th2 in remission MM patients were higher than that in non-remission patients. Some studies have suggested that Th2 cells may even promote tumor progression [9], but Th2 cells can promote the recruitment of tumoricidal eosinophils and macrophages into the tumor microenvironment and promote an antitumor immune response [19]. It is also recognized that Th2 cell-derived cytokines, such as IL-4 and IL-10, inhibit cell-mediated immunity [20], and elimination of CD4+ T cells, especially Th2 cells, enhances anti-tumor effect in mouse melanoma [21]. In our study, Th2 cells were significantly increased in MM patients, compared to healthy people, but the Tcm% of Th2 in remission MM patients were higher than in non-remission patients. This seems inconsistent. However, Tcms produce mainly IL-2, but after proliferation they efficiently differentiate into Tem, which produce large amounts of IL-4 to enhance antitumor effect [22]. That suggested elevated levels of Tcm of Th2 may be an indicator for remission of MM. Yet, the concrete mechanism by which T lymphocytes and associated cytokines are involved in the pathogenesis of MM remains unclear and merits further investigation. In summary, MM patients have reduced Th1 cells and increased the percentages of Th2 and Th17 cells. The changes of memory Th1 cells are related to bacterial infection in MM patients. Tcm of Th2 cells may be associated with the remission of MM. T-cell-related immunological dysfunction plays an important role in the pathogenic course of MM. However, the specific mechanism is not known and requires further study.

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Ethical Approval:
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Declaration of Interest:
The authors have no conflicts of interest to declare.

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