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ORIGINAL ARTICLE

Increased Number of Circulating Myeloid Dendritic Cell 1 in Patients with Severe Aplastic Anemia

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SUMMARY

Background: **A deeper understanding of the immune pathogenesis of severe aplastic anemia (SAA) is required to improve therapeutic effects. Myeloid dendritic cell (mDC) is involved in the initiation of immune disorders in SAA patients. The objective of this study was to characterize the subsets of mDC in patients with SAA.**
Methods: **A total of 136 SAA patients diagnosed in the Hematology Department of Tianjin Medical University General Hospital from December 2020 through November 2024 and 39 healthy controls were enrolled in this study. The percentages of the two main subsets of mDC (mDC1 and mDC2) in SAA patients with different disease status and healthy controls were detected by flow cytometry, and their correlations with the immune status and severity of SAA were analyzed.**
Results: **The mDC/plasmatoid dendritic cell (pDC) ratio of the untreated SAA group was significantly higher than that of the healthy control group (51.60 ± 122.16 vs. 4.77 ± 5.86, p = 0.015). MDC1 is the primary subset of mDC in all groups. The percentage of mDC1 in the untreated SAA group [(55.39 ± 22.99)%] was significantly higher than that in the partial remission group [(28.22 ± 26.37)%, p = 0.00], complete remission group [(25.55 ± 23.12)%, p = 0.00], and healthy control group [(19.22 ± 22.77), p = 0.00]. The percentage of mDC1 in the non-remission group [(40.25 ± 29.91)%] was significantly higher than that in the healthy control group [(19.22 ± 22.77)%, p = 0.026]. In the untreated SAA group, there was a significant negative correlation between the percentage of mDC1 and the reticulocyte count (r = -0.284, p = 0.048). There was no statistical difference in the percentage of mDC2 among the groups.**
Conclusions: **The activation of mDC1, rather than mDC2, might be involved in the pathogenesis of SAA. MDC1 intervention may have therapeutic potential in treating SAA.**
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KEYWORDS

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INTRODUCTION

Severe aplastic anemia (SAA) is a disease characterized by bone marrow failure and pancytopenia. Although the addition of Eltrombopag to intensified immunosuppressive therapy improved remission rate in SAA patients, some cases still had no response or relapse [1]. To improve outcomes, a deeper understanding of the pathogenesis of SAA is required.

It is currently believed that the polarization of T helper (Th)0 towards Th1, which results in the secretion of a large amount of negative regulators and the abnormal activation of cytotoxic T lymphocytes (CTL), constitutes the primary pathogenesis of SAA [2]. Our previous study demonstrated that myeloid dendritic cells (mDC) were implicated in the induction of immune responses in SAA patients by facilitating Th1 polarization [3]. Human mDC primarily consists of two subsets. Prototypic mDC, designated as mDC1, are $CD1c^{+}CD141^{-}$, accounting for approximately 50% of the total dendritic cell population in the periphery. MDC2 expresses $CD1c^{-}CD141^{+}$, comprising 5 - 10% of the population [4]. Although it has been previously confirmed that the total number of mDC is increased and their function is enhanced, little is known about the mDC subsets in SAA patients.

In this study, we extended our studies to detect the percentages of the two subsets of mDC in SAA patients with different disease status and healthy controls by flow cytometry and investigated the potential correlation between specific mDC subsets and the immune status and severity of SAA. It is expected that the results of this study will help to further refine the pathogenesis network of SAA and serve as the theoretical basis for establishing scientific and effective treatment plans for SAA.

MATERIALS AND METHODS

Subjects

A total of 136 SAA patients diagnosed in the Hematology Department of Tianjin Medical University General Hospital from December 2020 through November 2024 and 39 healthy controls were enrolled in this study. The diagnosis of SAA was compliant with 2016 International AA Study Group Criteria [5]. The treatment regimen for SAA patients was immunosuppressive therapy and Eltrombopag. Treatment efficacy was determined based on the Camitta standard published in 1979. We divided the participants into five groups: the untreated group, the non-response group, the partial response group, the complete remission group, and the healthy control group. In the untreated group, there were 49 cases, including 23 males and 26 females (median age 44, range 17 - 72). In the non-response group, there were 18 cases, including 12 males and 6 females (median age 34, range 18 - 65). In the partial response group, there were 46 cases, including 23 males and 23 females (median age 47, range 14 - 77). In the complete remission group, there were 23 cases, including 16 males and 7 females (median age 33, range 17 - 63). In the healthy control group, there were 39 cases, including 20 males and 19 females (median age 45, range 19 - 79). There was no significant difference in gender ($p = 0.309$) and age composition ($p = 0.088$) among the five groups. The study was approved by the Ethics Committee of the Tianjin Medical University. Informed written consent was obtained from all patients or their guardians in accordance with the Declaration of Helsinki.

Flow cytometric analysis

Bone marrow samples were collected by heparin anticoagulant tubes. APC-CD3, PE-CD4, and FITC-CD8 (BD Pharmingen) were used to label T-cell subtypes. The isotype controls were all from BD Pharmingen. The subsets of dendritic cells were measured using a DURACLONE IM Dendritic Cells Tube kit (Beckman Coulter) and the concentrations of Th-related cytokines were measured using a Human Th Assay kit (Celgene Biotech). The cytokines included Th1-related interleukin (IL)-2, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, Th2-related IL-4, IL-6 and IL-10, and Th-17 related IL-17. The experiment was carried out in strict accordance with the instructions provided by the manufacturer. Data acquisition was performed on a FACSCalibur system, and the acquired data were analyzed using CellQuest 3.1 software (Franklin Lakes).

Statistical analysis

The statistical analysis software SPSS 26 was used for data analysis. The gender composition was determined using the chi-squared test. The age composition was determined using the Kruskal-Wallis H-test. The one-way ANOVA was used to evaluate the differences among continuous variables normally distributed and a post-hoc Tukey test was used for multiple comparisons. The *t*-test was performed to evaluate the differences between two groups. Spearman's test was used for determining linear correlation of the data. Results with $p < 0.05$ were considered statistically significant.

RESULTS

The mDC/plasmatoid dendritic cell (pDC) DC ratio increased in untreated SAA patients

The mDC/pDC ratios of untreated SAA group, non-remission group, partial remission group, complete remission group, and healthy control group were 51.60 ± 122.16, 14.73 ± 19.80, 24.43 ± 37.86, 14.82 ± 20.64, and 4.77 ± 5.86, respectively. The mDC/pDC ratio of untreated SAA group was significantly higher than that of healthy control group ($p = 0.015$). There was no statistical difference between the other groups.

MDC1 is the main subset in mDC

In all groups, the percentages of mDC1 in mDC were significantly higher than those of mDC2, as shown in Table 1.

The percentage of mDC1 in mDC increased in untreated SAA patients

The percentage of mDC1 in mDC in untreated SAA group [(55.39 ± 22.99)%] was significantly higher than that in partial remission group [(28.22 ± 26.37)%, $p = 0.00$], complete remission group [(25.55 ± 23.12), p =

Table 1. The percentages of mDC1 and mDC2 subsets in each group.

	Percentage of mDC1 (%)	Percentage of mDC2 (%)	p-value
Untreated group	**55.39 ± 22.99**	**4.56 ± 5.98**	**0.000 ***
Non-remission group	**40.25 ± 29.91**	**4.52 ± 9.59**	**0.000 ***
Partial remission group	**28.22 ± 26.37**	**2.38 ± 3.09**	**0.000 ***
Complete remission group	**25.55 ± 23.12**	**1.87 ± 2.47**	**0.000 ***
Healthy control group	**19.22 ± 22.77**	**4.61 ± 14.83**	**0.000 ***

*** - $p < 0.05$.**

0.00], and healthy control group [(19.22 ± 22.77), p = 0.00]. The percentage of mDC1 in mDC in non-remission group [(40.25 ± 29.91)%] was significantly higher than that in healthy control group (p = 0.026). There was no statistical difference between the other groups. There was no statistical difference in the percentage of mDC2 in mDC among the groups.

The percentage of mDC1 correlated negatively with the count of reticulocyte in untreated SAA patients

In the untreated SAA group, there was a significant negative correlation between the percentage of mDC1 and the count of reticulocyte (r = -0.284, p = 0.048). No correlation was found between the percentage of mDC1 and other immune indicators (concentrations of IL-2, TNF-α, IFN-γ, IL-4, IL-6, IL-10 and IL-17, and $CD4^+$/$CD8^+$ ratio) or routine blood indices (count of red blood cell, hemoglobin, white blood cell, neutrophil, and platelet) (p > 0.05).

DISCUSSION

A study on the T cell receptor Vβ gene repertoire in AA patients revealed that their CTLs exhibited oligoclonal expansion characteristics. The result of high-throughput sequencing of Th1 cells in AA patients also demonstrated that their expansion possessed oligoclonality. These findings support the hypothesis of antigen-mediated T cell expansion in AA [6,7]. Dendritic cells play a pivotal role in antigen uptake and presentation. In response to antigen, mDCs secrete IL-12, controlling the activation of T cells to direct them into specific lineages and subsequently eliciting antigen-specific T cell immune responses [8]. Overactivation of mDCs can lead to inflammatory damage or autoimmune diseases [9-11].

Based on this foundation, our research group has conducted extensive work on mDC in patients with SAA. We previously showed that in newly diagnosed SAA patients, the total number of mDC was increased, the proportion of activated mDC was elevated, the expression of co-stimulatory molecules on mDC was up-regulated, and their phagocytosis and ability to stimulate lymphocytes were enhanced [3,12,13]. In this study, the mDC/pDC ratio of SAA patients in the untreated group was significantly higher than that in the healthy control group, which was consistent with previous studies. There was no statistically significant difference in the mDC/pDC ratio between the complete response group and the untreated group, suggesting that the recovery of mDC may take longer, which may be one of the reasons why clinical treatment needs to be maintained for a long time and the withdrawal of immunosuppressive agents needs to be slowed down. In previous studies, significant changes of mDC, showing levels lower than those before treatment, occurred only three years after intensified immunosuppressive therapy [14]. These findings suggest that abnormal activation of mDC may be an upstream factor in the immune pathogenesis of SAA.

Earlier studies have shown that mDC1 expresses high levels of major histocompatibility complex (MHC) II molecules and costimulatory molecules, possessing the specific ability to present antigens through MHC II to activate naive $CD4^+$ T cells and promote Th1 responses [4]. Additionally, mDC1 is more effective than mDC2 in promoting CTL responses, generating high levels of IFN-γ, granzyme B, and granzyme K [8]. MDC2, which is present in lower proportion than mDC1 in the circulation, primarily stimulates Th2 response. In this study, the percentage of mDC1 was significantly higher than that of mDC2 in all groups, suggesting that mDC1 is the dominant subset within mDC. The percentage of mDC1 was significantly elevated in newly diagnosed SAA patients, and this elevation could partially recover upon disease remission. In contrast, there was no statistically significant difference in the percentage of mDC2 among the groups. Furthermore, the percentage of mDC1 in untreated SAA patients was negatively correlated with their reticulocyte count, indicating that the percentage of mDC1 could reflect the severity of the disease to a certain extent. The increased mDC1, rather than mDC2, was more closely associated with the development of SAA. The increased mDC1 may be one of the initiating factors of the immune activation in SAA patients.

Due to their pivotal role as the bridge between innate and adaptive immune responses and their involvement in the pathogenesis of numerous autoimmune diseases, dendritic cells are considered potential targets for thera-

peutic modulation of immune responses. Therapies targeting dendritic cells represent a promising field in immunology, aiming to regulate cell function and thereby alleviate autoimmune diseases [15]. Many advances have been made in the treatment of autoimmune diseases through the modulation of dendritic cells using inhibitors targeting genes, proteins, cytokines, or through metabolic reprogramming [16-18]. We hypothesize that exploring the efficacy of dendritic cell-targeted therapies in SAA is also highly promising. Given that mDC1, which induces high expression of cytotoxic molecules in CTLs, is more abundant and particularly prevalent in untreated SAA patients, it is speculated that mDC1 may be a more promising candidate than mDC2. Our study had limitations; the sample-size was relatively small, particularly in the CR group, and several patients were not available during follow-up periods. Our future efforts will be focused on incorporating more patients to achieve a larger sample-size and thus improved statistics.

CONCLUSION

In this study, we provide a description of mDC subsets and their correlations with severity of SAA. MDC/pDC ratio and the percentage of mDC1 increased in untreated SAA patients, and the latter could partially recover upon disease remission. In addition, the percentage of mDC1 correlated negatively with the count of reticulocyte in untreated SAA patients. These findings highlight the important role of mDC1 in the induction of immune disorders in SAA patients, and mDC1 intervention may have therapeutic potential in treating SAA.

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Declaration of Interest:
The authors report that there are no competing interests to declare.

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