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Abnormal distribution of distinct lymphocyte subsets in children with Wiskott-Aldrich syndrome

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ABSTRACT

Wiskott-Aldrich syndrome (WAS) is a severe and rare primary immunodeficiency. Several studies show that WAS protein (WASp) plays a key role in the function of certain lymphocyte subsets. So far, no study has described distinct immunophenotypic abnormalities associated with WAS; thus the prognostic significance of any such abnormalities is unclear. This study examined many differences in the percentage/absolute numbers of distinct lymphocyte subsets in 20 WAS patients and 20 age/sex-matched healthy controls, and analyzed the association between these abnormalities and clinical disease scores. The results showed that the numbers of $CD4^+$ T cells, B cells, and $CD8^+$ naïve T cells were significantly lower in WAS patients; furthermore, the numbers in WASp-negative patients were lower than those in WASp-positive patients. WAS patients showed a selective reduction in expression of CD19 by naïve and transitional B cells. There was a negative association between the number of B cells and the WAS clinical scores. Also, $CD8^+$ naïve T cell numbers in patients with a score of 3–5A were lower than those in patients with a score of 2. The absence of WASp leads to a reduction in the population of specific lymphocyte subsets; therefore, these findings may help future management of patients with WAS.

1. Introduction

Wiskott-Aldrich syndrome (WAS) is a rare X-linked genetic immunodeficiency disorder characterized by thrombocytopenia and small platelets, eczema, and recurrent infections [1]. It is often associated with autoimmunity and an increased risk of malignancy. Although life expectancy of WAS patients is significantly reduced, hematopoietic stem cell transplantation is curative [2].

WAS is caused by mutations in the *WAS* gene on the X-chromosome; mutation impairs expression and/or function of the WAS protein (WASp) [3], a hematopoietic-specific regulator of actin nucleation that is also involved in signal transduction in various immune cells [4]. Several studies of WAS patients and WAS mouse models show that WASp plays a key role in the function of T cells, natural killer (NK) lymphocytes, and dendritic cells [5]. Impaired B cell compartment homeostasis has also been observed in WAS patients [6]. Previous studies examining the composition of the lymphocyte compartment in WAS patients describe reduced T cell numbers, but normal or only moderately suppressed B cell numbers [7].

The aim of the present study was to further investigate lymphocyte differentiation in WAS patients and its potential prognostic significance.

2. Materials and methods

2.1. Patients

A total of 20 patients harboring *WAS* mutations and twenty age/sex-matched healthy control subjects (HCs) were enrolled. Patients

Abbreviations: WAS, Wiskott-Aldrich syndrome; WASp, Wiskott-Aldrich syndrome protein; NK, nature killer lymphocytes; HCs, healthy control subjects; EM, effector memory; CM, central memory; TEMRA, terminally differentiated effector memory T; DNT, double negative T cells; MFI, mean fluorescence intensity.

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were diagnosed according to clinical symptoms, the absence/reduced expression of WASp, and the results of genetic analyses. Patients were assigned a score based on the clinical severity of the disease [8] (Table 1). HCs and patients were recruited from Children's Hospital of Chongqing Medical University, and the HCs had no related immune diseases and current infections. The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee at Children's Hospital of Chongqing Medical University.

2.2. Antibodies and reagents

The following antibodies were purchased from BD Biosciences (San Jose, CA): anti-human CD3, CD4, CD8, CD16, CD19, CD24, CD27, CD38, CD45, CD45RA, CD56, TCRαβ, TCRγδ, and IgD. Cell lysis solution was also purchased from BD Biosciences.

2.3. Flow cytometry

EDTA-anticoagulated fresh blood specimens were processed as described below. For the multi-color flow panel, each antibody was individually titrated and compared with its respective isotype control to determine the optimal dilution for a given volume (50 μl) of whole blood [9]. Briefly, the blood samples were split into three different panel fractions to discriminate specific cell populations: (a) the general lymphocyte population: B cells ($CD45^+$ $SSC^{low}CD19^+$), T cells ($CD45^+$ $SSC^{low}CD3^+$), CD4 T cells ($CD45^+SSC^{low}CD3^+CD4^+$), CD8 T cells ($CD45^+SSC^{low}CD3^+CD8^+$), and NK cells ($CD45^+SSC^{low}CD3^-CD56^+/CD16^+$),(b) T cell subsets: γδ T cell ($CD3^+TCR\gamma\delta^+$), naïve differentiated cytotoxic T lymphocyte (CD8 Naive, $CD3^+CD8^+CD45RA^+CD27^+$), terminally differentiated effector memory cytotoxic T lymphocyte (CD8 TEMRA, $CD3^+CD8^+CD45RA^+CD27^-$), central memory cytotoxic T lymphocyte (CD8 CM, $CD3^+CD8^+CD45RA^-CD27^+$), effector memory cytotoxic T lymphocyte (CD8 EM, $CD3^+CD8^+CD45RA^-CD27^-$), TCRαβ$^+$DNT ($CD3^+TCR\alpha\beta^+CD4^-CD8^-$), naive differentiated helper T lymphocyte (CD4 naive, $CD3^+CD4^+CD45RA^+CD27^+$), terminally differentiated effector memory helper T lymphocyte (CD4 TEMRA, $CD3^+CD4^+CD45RA^+CD27^-$), central memory helper T lymphocyte (CD4 CM, $CD3^+CD4^+CD45RA^-CD27^+$), and effector memory lymphocyte (CD4 EM, $CD3^+CD4^+CD45RA^-CD27^-$) subsets [10], and (c) B cell subsets: memory B cell ($CD19^+CD27^+IgD^+$), naive B cell ($CD19^+CD27^-IgD^+$), transitional B cell ($CD19^+CD24^{++}CD38^{++}$) and plasmablasts ($CD19^+CD24^-CD38^{++}$). For each sample, 50 μl whole blood was incubated with an antibody cocktail specific for the target cell population. After staining for 20 min at room temperature in the dark, erythrocytes in the samples were lysed by incubation with lysing solution for 5 min. Following centrifugation (500 g/2 min, room temperature) and washing with PBS, lymphocytes were fixed with 200 μl PBS containing 1% formaldehyde. Cells were then examined using a FACSCanto II flow cytometer (BD Biosciences) and data were analyzed using FACS Diva software.

2.4. Absolute values

The absolute number of lymphocytes in the samples was calculated by a hematologic analyzer (Sysmex xs-800i) using the following formula: absolute number of lymphocytes × % of lymphocyte subset = absolute number of cells in each subset.

2.5. Statistical analysis

Data are expressed as the mean ± SD and analyzed using a non-parametric (Mann–Whitney U) test. All statistical tests were performed in GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA). P values ≤ 0.05 were considered significant.

3. Results

3.1. Clinical findings in WAS patients

The demographic and diagnostic features of the WAS patients are shown in Table 2. The WASp expression were assessed by flow cytometric analysis and shown in Fig. S1. We quantified the lymphocyte subpopulations in these patients once they were diagnosed or during follow-up. The age of the patients at the time of participation in the study was 5 months to 4 years; the majority (16/20) were ≤2 years old. The clinical scores ranged from 2 to 5. Genetic mutations were analyzed by gene sequencing and included deletions, missense and nonsense mutations, and splice anomalies. WASp expression (as analyzed by flow cytometry) was absent in 60% (12/20) of cases.

3.2. Leukocyte and lymphocyte counts in WAS patients and HCs

The WAS patients and the age/sex-matched HCs were divided into subgroups on the basis of WASp expression. To calculate the absolute number of each specific cell subset, we first determined the overall leukocyte and lymphocyte counts (Fig. 1). Although the counts in some patients were lower than those in corresponding HCs, there was no statistical difference in the lymphocyte and leukocyte counts between groups.

3.3. Abnormalities in the distribution of major lymphocyte subsets

We next examined the distribution of major lymphocyte subsets (B cells, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and $CD3^-CD56^+/CD16^+$NK cells) in the peripheral blood of WAS patients and HCs. Again, WAS patients and HCs were divided into two groups based on WASp expression. The phenotypical characterization of the lymphocyte subsets is presented in Fig. 2.

The percentage and absolute number of T cells and $CD8^+$ T cells were no different between the groups (Fig. 2a). However, the percentage and absolute number of $CD4^+$ T cell and B cells were significantly lower in the WAS patients than in HCs. Notably, there was a significant reduction in $CD4^+$ T cells and B cells in WASp− patients compared to WASp+ patients (Fig. 2b). NK cell numbers were much

Table 1
WAS clinical scores.

Score	1	2	3	4	5
Thrombocytopenia	+	+	+	+	+
Small platelets	+	+	+	+	+
Eczema	−	(+)	+	++	(+)/+/++
Immunodeficiency	−/(+)	(+)	+	+	(+)/+
Infections	−	(+)	+	+/++	(+)/+/++
Autoimmunity and/or malignancy	−	−	−	−	+