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High-dose dexamethasone modulates serum cytokine profile in patients with primary immune thrombocytopenia

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ABSTRACT

Primary immune thrombocytopenia (ITP) is an autoimmune heterogeneous disorder which is characterized by decreased platelet count. Serum cytokines play an important role in the pathogenesis of ITP by initiating and perpetuating various cellular and humoral autoimmune processes. To investigate a broad spectrum of cytokines in ITP patients and the effects of high-dose dexamethasone (HD-DXM) regimen on serum cytokines profile, a multiplex cytokine assay was used to measure the serum levels of 20 circulating cytokines simultaneously in 22 patients before and after oral administration of 40 mg/day DXM for 4 consecutive days. A cohort of 10 healthy individuals was served as control. Serum levels of interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, growth-related oncogene (GRO), interferen (IFN)-γ and tumor necrosis factor (TNF)-α were significantly decreased in pre-treatment patients, compared with healthy controls ($p < 0.05$). After HD-DXM treatment, IL-4, IL-5, IL-6, IL-12p70, IL-13, GRO, IFN-γ and TNF-α were significantly increased in remission patients as compared with patients before treatment ($p < 0.05$). However, there was no significant difference (except TNF-α) between remission patients and healthy controls ($p > 0.05$). All these cytokines decreased again in relapse patients. Our findings suggest that measuring cytokine levels might help in the clinical assessment of ITP, and the HD-DXM therapy could correct the derangement of serum cytokines.

1. Introduction

Primary immune thrombocytopenia (ITP) is an autoimmune-mediated bleeding disorder characterized by decreased platelet count, with a considerable impact on quality of life [1,2]. The etiology of ITP remains unclear, but it is widely accepted that derangement of cellular immunity contributes to the pathogenesis of ITP [3]. While the autoantibodies produced by autoreactive B lymphocytes against platelet antigens and the dysfunctional cellular immunity to the disease have been acknowledged for many years, important aspects of the complex derangement of cytokines involved in the pathogenesis of ITP are still controversial. Cytokines, as molecular mediators in autoimmune diseases, play their role by binding specific cell receptors that either inhibit or induce cytokine-regulated genes [5]. Experimental data from various studies of ITP, including the clinical effectiveness of immunosuppressive therapy, strongly implicate T- and B-cells in the pathogenesis of the disease [4]. T helper 1 (Th1) and T helper 2 (Th2) are two functional subsets of helper T cells based on the types of their cytokine profiles. Th1 cells are characterized by the production of interferen (IFN)-γ and interleukin (IL)-2, and are important in the cell-mediated immunity. In contrast, Th2 cells synthesize IL-4, IL-5, IL-6, IL-10 and IL-13, and play a critical role in humoral immunity. Th1 and Th2 exert a mutual interplay through the antagonistic activity of their respective cytokines [6]. The imbalance of Th1 and Th2 subsets has a strong relationship with pathogenesis of a series of autoimmune diseases, including discoid lupus erythematosus (DLE) (Th1 dominant response) [7], experimental autoimmune encephalomyelitis (EAE) (Th1 dominant response) [8] and oral lichen planus (OLP) (Th2 dominant response) [9]. ITP has traditionally been classified as a Th1-mediated disease, such as Guo [10] and Wang [11]

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observed that ITP was characterized by Th1 cytokine polarization, whereas Andersson [12], Shariatmadar [13] and Webber [14] yielded inconsistent or opposing results. Cellular immunity function derangement has been demonstrated in ITP with abnormal cytokine profiles correlated to loss of immune tolerance [15].

Beside Th1 and Th2 cytokines, tumor necrosis factor (TNF)-α has previously been described as a crucial factor in the pathogenesis of autoimmune diseases [16,17]. Other cytokines, such as IL-12, IL-13 and GRO have also been described to have pathogenic role in autoimmune diseases [18,19]. As cytokines constitute a complicated network and contribute to the pathogenesis of a variety of diseases, circulating cytokines reflect the activation status of ongoing immune processes and the evaluation of serum cytokines is a good indicator and a reliable surrogate marker of disease activity in autoimmune diseases [20]. Moreover, cytokines differences were observed among patients responded to medicine [5]. To date, there are no generally accepted indicators to prognosticate ITP patients who are at risk of relapse, the common laboratory parameters are used for disease status evaluation, not for relapse prediction.

Among the various regimens, HD-DXM used in a short course is now recommended as an effective initial treatment for ITP patients [21]. Glucocorticoids are successfully used in the treatment of a wide range of diseases, exerting immunosuppressive, anti-inflammatory and anti-allergic effects on primary and secondary immune cells by different mechanisms [22,23]. Guo et al. [10] found that HD-DXM could correct Th1 polarization in ITP patients. With the light of these researches suggesting important role of cytokines in the pathogenesis of ITP, The aim of this study was to describe the expression profile of circulating cytokines in patients with ITP, so that could help the clinical assessment and to investigate the effect of HD-DXM on serum cytokine profile in ITP.

2. Materials and methods

2.1. Patients and controls

Twenty-two adult patients with newly diagnosed ITP were enrolled in this study (16 females and 6 males, median age 41 years, range 19–70 years, Table 1). These patients were treated and followed up at Zhongshan Hospital of Fudan University (Shanghai, China) between March 2010 and January 2013. All patients met the diagnosis criteria of ITP proposed by an international working group [3] and all required treatment due to clinically significant bleeding and/or extremely low platelet count. Secondary ITP, pregnant patients, and those who have complications with contraindications to glucocorticoid therapy were excluded. The control group consisted of 10 adult healthy volunteers (6 females and 4 males, median age 43 years, range 26–60 years). The study was approved by local Medical Ethics Committees of Zhongshan Hospital, Fudan University. Written informed consent was obtained from each patient before enrollment.

2.2. Treatment regimen

Patients with a platelet count of less than $20 \times 10^9\ L^{-1}$ or a platelet count of less than $50 \times 10^9\ L^{-1}$ and clinically significant bleeding received oral dexamethasone at a dose of 40 mg/day for four consecutive days. The response was evaluated according to the following criteria [3]: complete response (CR) was defined as a platelet count $\geq 100 \times 10^9\ L^{-1}$ and absence of bleeding; response (R) was defined as a platelet count ≥ 30 but $\leq 100 \times 10^9\ L^{-1}$ and a doubling from baseline and absence of bleeding; Loss of CR or R was defined as a platelet count below $100 \times 10^9\ L^{-1}$ or bleeding (from CR) or below $30 \times 10^9\ L^{-1}$ or less than 2-fold increase of baseline platelet count or bleeding (from R).

2.3. Measurement of cytokines

Peripheral blood samples were obtained from all patients before and after HD-DXM therapy. After being allowed to clot at room temperature for 1 h, peripheral blood sample were centrifuged at $1500 \times g$ for 10 min. All sera were stored at −80 °C until use. Serum levels of cytokines, including IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12(p70), IL-13, granulocyte-macrophage colony stimulating factor (GM-CSF), GRO, IFN-γ, CCL2 (monocyte chemoattractant protein (MCP)-1), CCL3 (macrophage inflammatory protein (MIP)-1α), CCL4 (MIP-1β), matrix metalloproteinase (MMP)-9, regulated upon activation normal T cell expressed and secreted factor (RANTES), vascular endothelial growth factor (VEGF) and TNF-α, were measured by Quantibody Human Cytokine Array 1 (RayBiotech, Norcross, GA, USA), following the manufacturer's specifications. Briefly, after 30-min incubation with sample diluent, the glass chips were washed, and each well, arrayed with cytokine antibodies, was overlaid with 100ul standard cytokines or sera. After overnight incubation at 4 °C and extensive washing, the detector antibody was added for 1 hour, washed away, and Cy3 equivalent dye-conjugated streptavidin was added for 1 hour at room temperature. The signals were scanned with a LuxScan-10K/A (Capitalbio, China). The amount of cytokine was quantified with Q-Analyzer Software (RayBiotech, Norcross, GA, USA) according to the standard curve set for each cytokine. In precaution of interassay variations, cytokines levels were measured using Quantibody array kit from the same shipment, and performed under the same laboratory conditions.

2.4. Statistical analysis

For statistical analysis, samples with cytokine levels below the detection limits were arbitrarily assigned the values corresponding to the minimum limits. Data were analyzed using SPSS 19.0 and GraphPad Prism 5.0 for Windows, and tested for normality using Kolmogorov–Smirnov normality test. Statistical significance of differences among the groups was determined by the one-way ANOVA (for parametric distribution), Kruskal–Wallis test and Mann–Whitney *U* test (for non-parametric distribution). In addition, Bonferroni-corrected post hoc test was conducted to adjust the observed significant level for multiple comparisons if the null hypothesis was rejected. Cytokine levels of patients before treatment were compared with their corresponding follow-up value at remission after HD-DXM therapy using Wilcoxon Signed Ranks test. Data are expressed as mean ± standard deviation (SD) and median. Patient characteristics were evaluated using chi-square analysis or the Fisher exact test. The p values less than 0.05 were considered to be significant.

3. Results

3.1. Patient characteristics

Twenty-two patients diagnosed with ITP were treated with HD-DXM (platelet count ranged between 1 and $21 \times 10^9\ L^{-1}$, with a median count of $8 \times 10^9\ L^{-1}$). Of the patients, twenty-two were CR or R by one week after the initiation of treatment (platelet count ranged between 94 and $387 \times 10^9\ L^{-1}$, with a median count of $189.5 \times 10^9\ L^{-1}$); ten were loss of CR or R during follow-up visits (platelet count ranged between 1 and $44 \times 10^9\ L^{-1}$, with a median count of $22.5 \times 10^9\ L^{-1}$). Characteristics of the twenty-two ITP cases (16 females and 6 males, median age 41 years, range 19–70 years) enrolled in this study are shown in Table 1. Ten matched healthy controls (6 females and 4 males, median age 43 years, range 26–60 years) platelet count ranged between 138 and $274 \times 10^9\ L^{-1}$, with