

Influence of Intravenous Immunoglobulin and Methylprednisolone on Cytokines secreted by T Lymphocyte in Children Primary Immune Thrombocytopenia¹

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Abstract

Objectives: Our study was designed to explore the Influence of Intravenous Immunoglobulin (IVIG) and Methylprednisolone (MP) on cytokines secreted by T lymphocyte in children Primary Immune Thrombocytopenia (ITP).

Materials and Methods: We enrolled children ITP at the onset of their disease from our department between December 2011 and March 2013. We followed them until 12 months of their whole duration, choosing the patients whose ultimate duration less than 3 months and no recurrence. Cytokines measurement by cytometric bead array included IL-2, IL-4, IL-6, IL-10, TNF, IFN and IL-17. We divided patients into 3 groups according to the treatment they received before testing cytokines level, then we compared T cell cytokines level among these groups.

Results: We enrolled 62 patients with first diagnosed ITP and ultimate duration was less than 3 months without recurrence (38 boys and 24 girls, age range 2-178 months, median 33 months). We found IL-2 level decreased in both groups treated with MP for 1 day (N = 29, P = 0.042) and group in which patients treated with IVIG for 1 day (N = 18, P = 0.048) compared with group without any treatment (N = 15).

Conclusion: As for children ITP, although pathways of MP and IVIG in treating thrombocytopenia were different, they both could decrease IL-2 in children ITP ultimately. As a representative cytokine of TH1 cell, IL-2 has been demonstrated to be important in lymphocyte activation and mobilization. So we considered that MP and IVIG can reduce the T cell activation and production of auto-antibodies by decrease IL-2.

Keywords: ITP; Cytokine; Children; Methylprednisolone; IVIG

Introduction

Primary Immune Thrombocytopenia (ITP) is characterized by independent thrombocytopenia, which is demonstrated as a disorder mediated by an immune mechanism. Children ITP often have duration of disease within 3 months. Most of them undergo a spontaneous remission, while the remains need treatment to control bleeding. However, there are still 20 - 30% patients who finally progress into persistent or chronic stage. Numerous studies prove that auto-antibodies against glycoproteins GPIIb/IIIa, GPIb/IX, etc. can be found in patients diagnosed with ITP. However, not all the ITP patients have positive results in auto-antibodies detection. There might be various mechanisms involved in the onset and progression of ITP. Current studies pay more attention to T lymphocyte abnormalities, including disturbance of TH1/TH2 balance. Studies have shown that patients suffering from ITP have polarized Th1 or Th2 response and cytokine deregulation. The Th1/Th2 balance was well known to regulate the immune system under normal

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conditions and to be impaired in autoimmune diseases like ITP. In order to study the mechanism of T lymphocyte abnormalities in children, ITP, we should ensure the homogeneity of the objects. Due to children ITP often have abrupt and severe onset of thrombocytopenia, we prescribe Intravenous Immunoglobulin (IVIG) at the first day of hospitalization to prevent hemorrhage, as well as Methylprednisolone (MP), which is often used when exclusion of malignancy. For this reason, T lymphocyte function evaluation involved detection of cytokines secreted by Th1, Th2 and Th17 etc might be interrupted by treatment we mentioned above. In this study, our objective was to explore the influence of IVIG and MP on cytokines secreted by T lymphocyte in children ITP.

Material and Methods:

Patients

We enrolled children, ITP at the onset of their disease without any treatment before hospitalization from Hematology and Oncology Center of Beijing Children Hospital between December 2011 and March 2013 in this prospective clinical study. The diagnosis of ITP was following International consensus report on the investigation and management of primary immune thrombocytopenia. We enrolled ITP patients whose disease duration were less than 3 months and age were between 2 months and 16 years. Patients diagnosed with an infection of Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), hepatitis virus and other pathogens which may potentially induce thrombocytopenia were ruled out. We followed them until 12 months of their whole duration, choosing the patients whose ultimate duration less than 3 months and no recurrence.

We divided them into 3 groups according to the treatment they received before testing cytokines level. Group 1 included patients who received neither MP nor IVIG. Group 2 included those who merely received MP (10mg/kg/d) for one day and Group 3 included those who merely received IVIG (0.4 g/kg/d) for one day. Informed consent was obtained from participating patients (when old enough) and their parents or guardians. Table 1

Table 1: Groups of different combination of treatment (IVIG and MP)

Groups	MP (days)	IVIG (days)	Cases of patients
GROUP 1	0	0	15
GROUP 2	1	0	29
GROUP 3	0	1	18

Legend: Group 1 included 15 patients who received neither MP nor IVIG. Group 2 included patients who merely received MP for 1 day. Group 3 included patients who merely received IVIG for 1 days.

Plasma Preparation

We collected blood of all the enrolled patients into evacuated tubes with 1.5mg/ml of Ethylenediaminetetraacetic acid (EDTA). All specimens were processed within 2 hrs of collection. Blood samples were centrifuged at 2000 rpm for 10 min to prepare the plasma. Levels of cytokines secreted by T lymphocyte were analyzed by cytometric bead array (CBA) using FACSCalibur Flow Cytometry from BD (Franklin Lakes, NJ). Cytokines included IL-2, IFN- γ and TNF which were mainly secreted by TH1 lymphocyte, IL-4, IL-6 and IL-10 which were mainly secreted by TH2 lymphocyte, IL-17 which were mainly secreted by TH17 lymphocyte. All procedures were carried out at room temperature.

Statistical Analysis

All statistical analyses were performed using SPSS version 21 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics including the mean and Standard Deviation (S.D) for quantitative variables and number and percentage for qualitative variables were performed. Differences in the cytokine levels among 3 groups were analyzed by One Way ANOVA. A p value of < 0.05 was considered to be statistically significant.

Results

Patients

62 patients of newly diagnosed ITP were enrolled in our study (38 boys and 24 girls, age range 2-178 months, median 33 months). The demographic and clinical characteristics of all enrolled children are summarized in Table 2.

Table 2: Patient's demographic and clinical characteristics.

Group	N.	Boy/Girl	Age (months)	Duration (days)	Min Plat (109/L)	HP positive	Bleed (No/Dry/Wet)
Total	62	38(61.3%) 24(38.7%)	33 (2 - 178)	3(1 - 42)	7(0 - 29)	7(11.3%)	5(8.0%) 49(79.0%) 8 (13.0%)

Levels of cytokines in groups of patients who received different days of IVIG and MP before testing cytokines level: We found IL-2 level decreased in both groups treated with MP for 1 day (N = 29, P = 0.042) and group in which patients treated with IVIG for 1 day (N = 18, P = 0.048) compared with group without any treatment (N = 15). Table 3

Table 3: Cytokines of children ITP patients with treatment of MP for 1 day, IVIG for 1 day and those without treatment before cytokines level been tested.

Parameter	No treatment(n=15)	MP for 1 DAY (n=29)	IVIG for 1 DAY (n=18)
IL-2	4.16±5.15	1.10±2.18 ^a *	1.07 ± 2.62 ^a *
IL-4	1.21 ± 1.28	0.84 ± 1.20	0.60 ± 0.95
IL-6	0.59 ± 0.67	0.64 ± 1.67	0.64 ± 0.85
IL-10	4.07 ± 3.66	4.31 ± 4.28	3.76 ± 2.64
TNF	2.78 ± 2.63	3.04 ± 2.95	2.60 ± 1.80
IFN	0.42 ± 0.63	0.34 ± 0.68	1.11 ± 2.60
IL-17	0.92 ± 0.93	0.58 ± 1.66	0.60 ± 0.72

a Significance compared to No treatment. *P<0.05 as statistically significant.

Discussion

ITP is a hemorrhagic disorder of thrombocytopenia without other secondary reasons which could cause decline of platelets. Researchers have found that autoimmune disturbances might be the possible reason for occurrence and development of ITP. Studies showed that patients with ITP demonstrate an increased Th1/Th2 ratio favouring auto-reactive B-cell development^[1]. As well as the Th17 cells produce cytokines such as IL-17 that may further drive the imbalance between Th1 and Th2 cells, therefore favouring autoimmunity^[2]. As for children patients, majority of them will remiss within 3 months, while 20-30% of them develop into a chronic form and have poor life-quality^[3]. It is eager for us to research in the factors related to the prognosis of children ITP, esp. the immune factors. In order to obtain results with high credibility, we need to exclude the influence of the treatment to the immune factors like cytokines which secreted by T lymphocytes. The standard first-line therapy of ITP was MP and IVIG in our children hospital. So the objective of our study was to explore the influence of IVIG and MP on cytokines secreted by T lymphocyte in children ITP.

MP in symptomatic childhood ITP patients has been suggested as an appropriate first-line therapy^[4-7] and it seems wise to use high-dose regimens for as short a period of time as is necessary to achieve a clinically meaningful endpoint^[8]. MP is a kind of glucocorticoid that prevents the release of substances in the body that cause inflammation. It can mechanically activate the cytosolic glucocorticoid receptor and thus inhibit the pro-inflammatory transcription factor, nuclear factor kappa B (NF-kB). These interactions are important in anti-inflammatory effects of glucocorticoids^[9]. MP can suppress inflammation by decreasing the release of pro-inflammatory cytokines and vasoactive factors. According to package inserts of SOLU-MEDROL (MP sodium succinate), it has a greater anti-inflammatory potency than prednisolone. After an intravenous infusion of MP, 30 mg/kg over a 20 minute period, peak plasma concentrations of approximately 20 mcg/mL were achieved. The mean elimination half-life of MP ranges from 1.8 to 5.2 hours.

IVIG has been reported by Paul Imbach in 1981 that it could increase platelet counts in ITP patients and is considered as their standard therapy^[10]. IVIG may increase platelet counts in ITP by decreasing FcγR-mediated platelet destruction via competitive-inhibition of Fcγ receptors (FcγR), inhibiting complement-mediated platelet elimination, increasing anti-platelet antibody clearance through saturation FcRn, etc^[11-12]. Rong Deng in 2007 has proved that 43 ± 5% of overall effects of IVIG on MWRReg30 (anti-platelet antibody) induced thrombocytopenia in mice could be accounted for by the IVIG-mediated increases in MWRReg30 clearance^[13].

In this study, we found that MP and IVIG both could influence the levels of IL-2. Patients who received MP and IVIG for 1 day had lower levels of IL-2 than those who didn't receive MP. The results proved that although pathways of MP and IVIG in treating thrombocytopenia were different, they both could decrease IL-2 in children ITP ultimately. Several studies have found evidence supporting a Th1 polarization of the immune response in ITP^[14-16]. Several studies have shown the presence of activated platelet-specific autoreactive T cells that recognize and respond to autologous platelet antigens and drive the generation of platelet reactive autoantibodies by B cells in the peripheral blood of ITP patients^[17-19]. As a representative cytokine of TH1 cells, IL-2 has been demonstrated to be important in lymphocyte activation and mobilization^[20]. So we considered that although pathways of MP and IVIG in treating thrombocytopenia were different, they both could decrease IL-2 in children ITP ultimately. In other words, they both could suppress the activation of T cell and thus re-adjust the TH1/TH2 imbalance, inhibit the destruction lead by T cell and the generation of antibodies by activated B cell.

In conclusion of our study, as for children, ITP, MP and IVIG for at least 1 day would change patterns of cytokines, esp. level of IL-2 decreased after using MP and IVIG, but the mechanism still need to be prove. Furthermore, we should enlarge our sample size, enroll healthy children as the control group and try our best to acquire datum of the same patients before and after the treatment.

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