J Clin Immunol (2007) 27:557–562
DOI 10.1007/s10875-007-9111-1

Correction of Th1-dominant Cytokine Profiles by High-dose Dexamethasone in Patients with Chronic Idiopathic Thrombocytopenic Purpura

Chengshan Guo · Xiaoxia Chu · Yan Shi · Weidong He · Lizhen Li · Lin Wang · Yingxue Wang · Jun Peng · Ming Hou

Received: 2 March 2007 / Accepted: 6 June 2007 / Published online: 6 July 2007

Abstract To investigate the possible correcting of T helper (Th) cytokine profiles by high-dose dexamethasone (HD-DXM) therapy in chronic idiopathic thrombocytopenic purpura (ITP) with active disease, we determined the plasma levels of IFN-γ, IL-2, IL-4, IL-10, and TGF-β1 in 52 patients before and after oral administration of 40 mg/day DXM for four consecutive days. The cytokine levels were measured by enzyme-linked immunosorbent assay. The results showed that initial responses were reached in all patients and sustained response (SR) rate is 46.15%. The pretreatment plasma levels of both IFN-γ and IL-2 were significantly increased and those of IL-4, IL-10, and TGF-β1 significantly decreased, compared with those of the normal controls ($P<0.01$), indicating a Th1-dominant cytokine profile typically found in ITP. After HD-DXM treatment, IFN-γ and IL-2 were decreased ($P<0.01$), whereas IL-4 and IL-10 were increased ($P<0.05$). There was no significant difference between the HD-DXM-treated patients and the normal controls ($P>0.05$). TGF-β1 was also increased ($P<0.01$) after HD-DXM treatment, but still lower than that of the normal controls ($P<0.05$). During following-up, the cytokine profiles in the SRs remained stable compared to the posttreatment level ($P>0.05$), but IFN-γ and IL-2 levels raised up, and IL-4, IL-10, and TGF-β1 levels reduced again in the relapsed patients ($P<0.01$). Our data demonstrate that HD-DXM is an effective initial therapy for ITP, and the Th1 cytokine dominance could be corrected by HD-DXM.

Keywords Idiopathic thrombocytophenic purpura · cytokines · T helper lymphocyte · dexamethasone

C. Guo · Y. Wang
Department of Hematology,
Second Hospital of Shandong University,
Jinan 250033, China

X. Chu
Department of Hematology, Yantai Yuhuangding Hospital,
Yantai 264000, China

Y. Shi · L. Li · L. Wang · J. Peng · M. Hou (✉)
Department of Hematology, Qilu Hospital, Shandong University,
Jinan 250012, China
e-mail: houming@medmail.com.cn

W. He
Shandong Blood Center,
Jinan 250014, China

Introduction

Chronic idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder manifested by antiplatelet autoantibody-mediated thrombocytopenia. Like in many other autoimmune diseases, autoantibody production by B cells in ITP needs the help of T cells, as evidenced by its association with both T cell activation and T–B cognate interaction [1–4]. Almost all of these autoantigen-specific T cells are $CD4^+$ T helper (Th) cells, which may play very important roles in the pathogenesis of ITP [5–9]. Recent investigations have substantiated a Th1 polarization of the immune response in ITP [10, 11].

Although the best therapeutic approach is not yet unanimous, prednisone or prednisolone has been widely

recognized as the most appropriate front-line therapy for most ITP patients who need to be managed. In recent years, some reports showed that high-dose dexamethasone (HD-DXM) might be a promising alternative to prednisone as the first- or second-line treatment in ITP patients [12–14]. In this study, oral HD-DXM was used in a single 4-day course as the initial treatment schedule in previously untreated ITP patients with active disease, and the plasma Th1, Th2, and Th3 cytokines were profiled in these patients before and after HD-DXM treatment.

Materials and Methods

Patients

Fifty-two ITP patients with active disease (34 females and 18 males, age range 13–73 years, median 39 years) were enrolled in this study. Enrollment took place between April 2003 and January 2005 at the Hematology Department of Qilu Hospital and the Second Hospital affiliated to Shandong University, Jinan, China. The patients' platelet count ranged between 1 and 49×10^9/l, with a median count of 13.5×10^9/l, all required treatment because of clinically significant bleeding. All of the cases met the diagnosis criteria of chronic ITP as previously described [15, 16]. None of them had been treated with glucocorticosteroid previously. Patients complicated with diabetes, hypertension, cardiovascular diseases, pregnancy, active infection, or connective tissue diseases, such as systemic lupus erythematosus, were excluded.

The control group consisted of 20 adult healthy volunteers (13 females and 7 males, age range 22–51 years, median 31 years). Platelet counts were ranged from 150 to 342×10^9/l, with the median count of 207×10^9/l.

The study was approved by the Medical Ethical Committee of Qilu Hospital and the Second Hospital of Shandong University. Informed consent was obtained from each patient before being included in the study.

Treatment Regimen

Dexamethasone (DXM) 40 mg/day was administered orally for four consecutive days. No maintenance or other treatment modality was used. Initial response evaluation was made at the end of the second week after treatment initiation. The response was evaluated according to the following criteria: complete response (CR) defined as platelet count $\geq150\times10^9$/l; partial response (PR) defined as platelet count $\geq50<150\times10^9$/l; minimal response (MR) defined as platelet count $\geq30<50\times10^9$/l, and cessation of bleeding; no response defined as platelet count $<30\times10^9$/l or persistence of bleeding symptoms related to thrombocytopenia; and sustained response (SR) was defined as platelet count remained $\geq30\times10^9$/l and cessation of bleeding in 6 months of follow-up, otherwise it was defined as relapse.

Laboratory Assay

Complete blood counts were performed at recruitment, on day 15 after HD-DXM treatment, and during follow-up period. Bone marrow aspiration was performed routinely at recruitment. Blood glucose levels were measured at recruitment and after treatment with HD-DXM.

Cytokine Assay

All of the plasma samples were isolated from 10-ml ethylenediaminetetraacetic acid (Pharmacia, USA)-anticoagulated peripheral blood by centrifuging at 2,000 rpm at room temperature (RT) for 20 min twice, and then stored at −20°C for future use. Plasma samples were obtained before therapy and 2 weeks after therapy. In addition, out of all patients' post-treatment, 33 cases including 12 SR and 21 relapsed cases, respectively, were also examined of the plasma cytokine levels at the median time of 6 months during following-up.

Plasma levels of IFN-γ, IL-2, IL-4, IL-10, and TGF-β1 were measured by enzyme-linked immunosorbent assay (ELISA, kits from R&D Systems Inc, USA) in 96-well plates for both the test group and the standard group, whereas the standard group and the blank were duplicated following instructions of the assays. All reagents, standards, and samples were prepared according to the manufacture's instructions. Microplate strips were removed from the plate frame, and 100 μl of Assay Diluent RD1S was added to each well except blank contrast ones, and incubated for 2 h at RT. A plate layout was provided to record the standards and samples assayed. Followed by aspirating and washing with Wash Buffer (Sigma, USA) for four times. Two hundred microliters of conjugate was added to each well and incubated for 2 h at RT, then aspirated and washed as above. As followed, 200 μl of substrate solution was added to each well and incubated for 30 min at RT while protected from light. Finally, 50 μl of stop solution was added to each well and data was read at 450 nm with automatic ELISA analysis apparatus (version 5.0.1 Build 52 software system, BIO-RAD Systems Inc., USA).

Statistical Analysis

Data were expressed as mean ± SD. The differences between two groups and patients before and after treatment were evaluated using unpaired and paired Student *t* test, respectively. All tests were performed by SPSS 13.0 system.

Table I Clinical Characteristics and Initial Responses of ITP Patients

Patient no.	Sex/Age (year)	Time from diagnosis to treatment (month)	Bleeding symptoms	Platelet counts ($\times 10^9$/l)	
				Pretreatment	Posttreatment
1	F/17	6	PT, EC, EP	8	364
2	F/46	8	EC	22	78
3	F/48	9.5	PT, EC	17	43
4	M/47	1	EC, GH	7	332
5	M/59	4	EC, EP	3	240
6	F/39	11	PT	12	86
7	F/36	3	EC, GUH	17	233
8	M/19	6	EC	4	247
9	F/77	2	EP	42	58
10	F/41	2	PT	25	170
11	M/73	8.5	EC	9	148
12	F/60	1	PT	24	101
13	F/41	8.5	EC, GH	6	230
14	F/39	1	PT, EC	11	92
15	M/42	8.5	EC	15	181
16	F/26	6.5	EC, GUH	23	156
17	M/41	6	PT, GH	4	121
18	M/38	2	PT, EC	14	143
19	F/15	2.5	EC, GH	5	179
20	F/54	2	PT	12	46
21	M/13	2	EC	8	150
22	F/40	2.5	EC, GUH	18	87
23	F/27	3	PT	16	130
24	M/41	8	PT	16	160
25	M/25	6	EC	22	41
26	F/66	2.5	PT	20	143
27	F/38	5.5	PT	22	155
28	F/31	4	PT, GUH	11	231
29	F/50	6.5	EC	8	165
30	M/35	6	EC, GUH	15	310
31	F/29	6	EC, EP	1	237
32	F/21	3	EC	17	91
33	M/30	7	PT	20	328
34	F/63	2	EC, GH	1	120
35	F/13	5.5	PT	26	363
36	M/50	4.5	PT	47	130
37	F/16	8.5	EC, EP	5	124
38	F/35	3	EC, GUH	3	94
39	F/20	4	PT, EC	7	186
40	F/52	6.5	PT, EC	13	157
41	F/17	4	PT	29	224
42	M/41	9	PT	13	162
43	F/51	4.5	EC	16	185
44	F/20	6	PT	12	194
45	F/30	4	EC, GUH	18	79
46	M/58	9	EC, GH	10	387
47	F/59	1	EC, GIH	4	138
48	M/50	8.5	EC	49	192
49	F/26	11	E, GUH	12	108
50	F/23	1	PT	14	280
51	M/45	30	EC	20	155
52	M/36	10	PT	32	47
Median (min–max)	39 (13–73)	5.5 (1–30)		13.5[a] (1–51)	152.5[b] (41–387)

PT = Petechiae, EC = ecchymoses, EP = epistaxis, GUH = genitourinary hemorrhage, GH = gingival hemorrhage, GIH = gastrointestinal hemorrhage

[a] $P<0.01$, ITP pretreatment vs normal control

[b] $P<0.01$, ITP posttreatment vs pretreatment

Results

Clinical Therapeutic Effect of HD-DXM

Responses were reached in all patients: CR in 29 (55.77%), PR in 19 (36.54%), and MR in 4 (7.69%). Median platelet count was 152.5×10^9/l (min–max 41–387), and only four patients had a platelet count of less than 50×10^9/l after HD-DXM treatment (Table I). No bleeding or other obvious complications was observed throughout the treatment.

Side effects No serious side effects of glucocorticosteroids occurred, such as metabolized abnormality of multiple systems and withdrawal symptoms; blood glucose level increased temporarily in two patients and insomnia occurred in two patients during short-term DXM therapy, therefore HD-DXM therapy was well tolerated.

Follow-up The median follow-up period was 6 months (range 5.5–12 months). Among all of the 52 patients, 24 (46.15%) had SR with platelet count of more than 30×10^9/l after a single course of HD-DXM without any further therapy during follow-up. The remaining 28 patients (53.85%) had a relapse within 6 months that required additional treatment.

Cytokine Changes in ITP Patients

Compared with the normal controls, in ITP patients with active disease, the plasma levels of both IFN-γ and IL-2 were increased significantly ($P<0.01$). On the contrary, IL-4, IL-10, and TGF-β1 levels were considerably decreased ($P<0.01$) (Table II).

Cytokine Profile Correction After Treatment

Both of IFN-γ and IL-2 levels were decreased significantly ($P<0.01$) and normalized after HD-DXM treatment. The levels of both IL-4 and IL-10 were increased significantly ($P<0.01$) after treatment. There was no significant difference between the treated patients and the normal controls ($P>0.05$). TGF-β1 was increased significantly ($P<0.01$) after treatment, but was still lower than that of the normal controls ($P<0.05$) (Table II).

The cytokine profiles in the SRs remained stable compared to the posttreatment level ($P>0.05$). On the contrary, IFN-γ and IL-2 levels raised up, and IL-4, IL-10, and TGF-β1 levels reduced again in the relapsed patients ($P<0.01$) (Table II).

Discussion

In our study, all of the patients exhibited initial responses to 4 days of HD-DXM treatment. Remarkably, more than half of the patients (55.77%) reached CR, while only four patients (7.69%) had posttreatment platelet counts of less than 50×10^9/l. Even more remarkable, 46.15% of the ITP patients obtained SR that did not need additional treatment during the 6-month follow-up. The patients' compliance, efficacy, and safety profile of the 4-day HD-DXM treatment in the present study was satisfactory compared with usual prednisone or prednisolone scheme, supporting the proposal of HD-DXM as an alternative initial therapy for ITP [12–14, 17, 18].

It has been described that depletion of $CD8^+$ T cells with anti-CD8 monoclonal antibodies and complement did not reduce the proliferative capacity of the responding peripheral blood mononuclear cells from patients with ITP, indicating that $CD4^+$ T helper cells may be responsible for the response [5]. A recent study assessed the intracellular IL-4 and IFN-γ production in $CD4^+$ T lymphocytes activated by phorbol 12-myristate 13-acetate and ionomycin in patients with ITP, and found that the Th1/Th2 ratio in the untreated group was significantly higher than that in the control group [10]. In accordance with previous reports [10,

Table II Plasma Cytokine Levels in ITP Patients' Pre- and Posttreatment, SR, and Relapse

Cytokines	Normal control ($n=20$)	Pretreatment ($n=52$)	Posttreatment ($n=52$)	SR ($n=12$)	Relapsed ($n=21$)
IFN-γ (pg/ml)	10.23±3.97	22.71±7.98[a]	11.57±4.33[b]	12.42±4.01	21.02±8.63[c,d]
IL-2 (pg/ml)	8.73±8.22	28.42±11.27[a]	14.56±10.76[b]	11.77±8.95	23.55±12.91[c,d]
IL-4 (pg/ml)	14.39±8.03	5.93±3.85[a]	9.87±4.82[e]	11.03±5.64	5.29±2.47[c,d]
IL-10 (pg/ml)	8.67±3.04	3.24±1.36[a]	7.90±2.71[b]	8.15±2.83	4.37±2.62[c,d]
TGF-β1 (ng/ml)	7.87±2.54	1.31±0.71[a]	4.19±1.80[b,f]	6.36±3.43	1.89±0.76[c,d]

[a] $P<0.01$, ITP pretreatment vs normal control
[b] $P<0.01$, ITP posttreatment vs pretreatment
[c] $P<0.01$, ITP in relapsed vs SR
[d] $P<0.01$, relapsed vs posttreatment
[e] $P<0.05$, ITP posttreatment vs pretreatment
[f] $P<0.05$, ITP posttreatment vs normal control

19, 20], the plasma levels of IFN-γ, IL-2, IL-4, and IL-10 in the patients included in the present study further implied a Th1-dominated cytokine profile in ITP with active disease. In addition, our data show that TGF-β1 concentration varied inversely with the disease activity; the significantly lower level of TGF-β1 observed in this cohort should be associated with a downregulated Th3 response in active ITP patients, but it significantly elevated after HD-DXM management, so this suggest that Th3 may play an important role in bystander immune suppression in ITP [21–23].

More interestingly, we found that HD-DXM therapy for ITP could cause a shift in the Th1/Th2 cytokine balance to the same levels as normal controls, leading to a more balanced Th1/Th2/Th3 cytokine profile response in vivo [10, 24, 25]. Glucocorticoids (GC) were known to affect cytokine synthesis in T cells by binding to and activating cytoplasmic GC receptors. The receptor–corticosteroid complex then translocates to the nucleus, where it regulates the transcription of target genes through several mechanisms [4, 26, 27]. GC may directly inhibit Th1 cytokine production in T cells and potentially enhance Th2 cytokine synthesis by inhibiting IL-12 production in antigen-presenting cells [28]. The HD-DXM-mediated Th1/Th2 cytokine profile alterations observed in this study could be the results of a down-regulation of Th1 cytokines while permitting the production of Th2 cytokines. The precise mechanisms of HD-DXM await further elucidation. Furthermore, our data displayed that in the patients who maintained the response could also sustain their cytokine profiles very much resembling their posttreatment pattern; however, the cytokine profiles tended to get back to the baseline values in the relapsed patients. These data indicated that relapsed ITP rooted in recurring of the Th cytokine imbalance, and may need repeated or additional treatment.

In summary, our results support that ITP is a Th1-predominant autoimmune disease and a 4-day course of HD-DXM is an effective and safe initial therapy for ITP. The Th1 polarization could be corrected by HD-DXM therapy, which might be a chief mechanism of HD-DXM immunotherapy for ITP.

Acknowledgments We are grateful to the following scholars for their clinical and laboratory help: Prof. Zhenhua Tu, Prof. Chunyan Ji, Yuanyuan Zhu, Prof. Luqun Wang, Dr. Chuanli Zhao, and Dr. Qiang Song (Centre of Haematol/Oncology, Qilu Hospital, Shandong University, Jinan, China), and we are also grateful to Dr. Qianli Zhuang for critical review of this article. This work is supported by grants from National Natural Science Foundation of China (nos. 30300312, 30470742, 30579779, and 30600259), 973 Program 2006 CB 503803 and Key Clinical Research Project of Public Health Ministry of China 2004–2006.

References

1. Kuwana M, Kaburaki J Ikeda Y. Autoreactive T cells to platelet GPIIb-IIIa in immune thrombocytopenic purpura: role in production of anti-platelet autoantibody. J Clin Invest 1998;102:1393–402.
2. Hedlund-Treutiger I, Wahlstrom J, Elinder G. Role of the T cell receptor in idiopathic thrombocytopenic purpura (ITP). Acta Paediatr Suppl 1998;424:46–50.
3. Semple JW, Lazarus AH, Freedman J. The cellular immunology associated with autoimmune thrombocytopenic purpura: an update. Transfus Sci 1998;19:245–51.
4. Cines DB, McMillan R. Management of adult idiopathic thrombocytopenic purpura. Annu Rev Med 2005;56:425–42.
5. Semple JW, Freedman J. Increased antiplatelet T helper lymphocyte reactivity in patients with autoimmune thrombocytopenia. Blood 1991;78:2619–25.
6. Garcia-Suarez J, Prieto A, Reye E, Manzano L, Merino JL, Alvarez-Mon M. The clinical outcome of autoimmune thrombocytopenic purpura is related to their T-cell immunodeficiency. Br J Haematol 1993;84:464–70.
7. Ware RE, Howard TA. Elevated numbers of gamma delta(γδ+)T lymphocytes in children with immune thrombocytopenic purpura. J Clin Immunol 1994;14:237–47.
8. Crossley AR, Dickinson AM, Proctor SJ, Calvert JE. Effects of interferon-α therapy on immune parameters in immune thrombocytopenic purpura. Autoimmunity 1996;24:81–100.
9. Erduran E, Aslan Y, Aliyazicioglu Y, Mocan H, Gedik Y. Plasma soluble interleukin-2 receptor levels in patients with idiopathic thrombocytopenic purpura. Am J Hematol 1998;57:119–23.
10. Ogawara H, Handa H, Morita K, Hayakawa M, Kojima J, Amagai H, et al. High Th1/Th2 ratio in patients with chronic autoimmune thrombocytopenic purpura. Eur J Haematol 2003;71:283–8.
11. Panitsas FP, Theodoropoulou M, Kouraklis A, Karakantza M, Theodorou GL, Zoumbos NC, et al. Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. Blood 2004;103:2645–7.
12. Wali YA, Al Lamki Z, Shah W, Zacharia M, Hassan A. Pulsed high-dose dexamethasone therapy in children with chronic idiopathic thrombocytopenic purpura. Pediatr Hematol Oncol 2002;19:329–35.
13. Cheng Y, Wong RS, Soo YO, Chui CH, Lau FY, Chan NP, et al. Initial treatment of immune thrombocytopenic purpura with high-dose dexamethasone. N Engl J Med 2003;349:831–6.
14. Borst F, Keuning JJ, van Hulsteijn H, Sinnige H, Vreugdenhil G. High-dose dexamethasone as a first- and second-line treatment of idiopathic thrombocytopenic purpura in adults. Ann Hematol 2004;83:764–8.
15. George JN, Woolf SH, Raskob GE. Idiopathic thrombocytopenic purpura: a guideline for diagnosis and management of children and adults. American Society of Hematology. Ann Med 1998;30:38–44.
16. British Committee for Standards in Haematology. General Haematology Task Force: Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. Br J Haematol 2003;120:574–96.
17. Gutierrez-Espindola GR, Morales-Polanco MR, Guerrero-Rivera S, Talavera JO, Sanchez-Valle E, Gomez-Morales E, et al. High doses of dexamethasone in adult patients with idiopathic thrombocytopenic purpura. Arch Med Res 2003;34:31–4.
18. Mazzucconi MG, Fazi P, Bernasconi S, De Rossi G, Leone G, Gugliotta L, et al. Therapy with high-dose dexamethasone (HD-DXM) in previously untreated patients affected by idiopathic thrombocytopenic purpura. A GIMEMA experience. Blood 2007;109 (4):1401–7.

19. Andersson J. Cytokines in idiopathic thrombocytopenic purpura (ITP). Acta Paediatr Suppl 1998;424:61–4.
20. Yoshimura C, Nomura S, Nagahama M, Ozaki Y, Kagawa H, Fukuhara S. Plasma-soluble Fas (APO-1, CD95) and soluble Fas ligand in immune thrombocytopenic purpura. Eur J Haematol 2000;64:219–24.
21. Andersson PO, Stockelberg D, Jacobsson S, Wadenvik H. A transforming growth factor-beta1-mediated bystander immune suppression could be associated with remission of chronic idiopathic thrombocytopenic purpura. Ann Hematol 2000;79: 507–13.
22. Andersson PO, Olsson A, Wadenvik H. Reduced transforming growth factor-beta1 production by mononuclear cells from patients with active chronic idiopathic thrombocytopenic purpura. Br J Haematol 2002;116:862–7.
23. Jackson M, Ahmad Y, Bruce IN, Coupes B, Brenchley PE. Activation of transforming growth factor-beta1 and early atherosclerosis in systemic lupus erythematosus. Arthritis Res 2006;8:R81.
24. Agarwal SK, Marshall GD Jr. Dexamethasone promotes type 2 cytokine production primarily through inhibition of type 1 cytokines. J Interferon Cytokine Res 2001;21:147–55.
25. Wang T, Zhao H, Ren H, Guo J, Xu M, Yang R, et al. Type 1 and type 2 T-cell profiles in idiopathic thrombocytopenic purpura. Haematologica 2005;90:914–23.
26. Adcock IM, Brown CR, Gelder CM, Shirasaki H, Peters MJ, Barnes PJ. The effects of glucocorticoids on transcription factor activation in human peripheral blood mononuclear cells. Am J Physiol 1995;37:C331.
27. Peng J, Liu CF, Liu D, Ren C, Li W, Wang Z, et al. Effects of B7-blocking agent and/or CsA on induction of platelet-specific T-cell anergy in chronic autoimmune thrombocytopenic purpura. Blood 2003;101:2721–6.
28. DeKruyff RH, Fang Y, Umetsu DT. Corticosteroids enhance the capacity of macrophages to induce Th2 cytokine synthesis in $CD4^+$ lymphocytes by inhibiting IL-12 production. J Immunol 1998;160:2231–7.