Thrombosis Research 140 (2016) 73–80

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Efficacy of immunomodulatory therapy with all-*trans* retinoid acid in adult patients with chronic immune thrombocytopenia☆

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ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 22 January 2016
Accepted 12 February 2016
Available online 15 February 2016

Keywords:
Chronic immune thrombocytopenia
All-*trans* retinoid acid
Regulatory T cells
Immunomodulatory therapy

ABSTRACT

Introduction: Immune thrombocytopenia (ITP) is a common hematologic disorder characterized by isolated thrombocytopenia. In adults, ITP is more likely to be chronic, requiring individualised treatment and management. Corticosteroids and splenectomy are the most common therapy for ITP. However, these routine approaches failed in these patients with chronic ITP. The aim of this study was to evaluate the efficacy of immunomodulatory therapy with all-*trans* retinoid acid (ATRA) in adult patients with chronic ITP.
Materials and methods: ATRA therapy was applied in a total of 35 patients with chronic ITP who failed with standard dose corticosteroids and/or splenectomy. The response ratio and the change of the T cell subsets including Th1, Th2, Th17 and Treg, were evaluated.
Results: Complete response and overall response were observed in 10 (28.6%) and 19 patients (54.3%), respectively. Compared with the control group, a significant decreased level of Treg cells, IL-10 and Foxp3 expression were found in ITP patients. ATRA therapy could significantly increase the percentage of Treg cell, IL-10 level and Foxp3 expression.
Conclusions: Our findings indicate that ATRA therapy could induce significant changes of Treg cells to induce response in patients with chronic ITP.

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1. Introduction

Chronic immune thrombocytopenia (ITP), an autoimmune disease, is characterized by reduced platelet counts and a variably increased risk of bleeding. A high risk of death and disease-related or therapy-related complications could be presented in patients with chronic ITP [1,2]. Treatment options of these patients include aggressive immunosuppressant agents, and most recently thrombopoietin (TPO) receptor agonists [3,4]. Moreover, single-agent immunosuppressant drugs such as azathioprine and cyclosporine were also used to treat patients with moderate success [5]; however, dose escalation to achieve treatment efficacy can also cause morbidity. Therefore, other therapeutic options are needed.

All-*trans* retinoic acid (ATRA), a vitamin A metabolite, has been shown to be involved in a wide range of biological processes, including cell proliferation and differentiation [6]. Previous studies have been shown to be effective in treatment of acute promyelocytic leukemia (APL) through differentiation induction and terminal cell division of leukemia cells [7–9]. Recent studies revealed that ATRA promotes the development and function of T helper (Th) cells and Foxp3+ Treg cells [10,11]. However, the effectiveness of ATRA in the treatment of chronic ITP remains unknown.

Here, we firstly conducted a prospective study to investigate the efficacy of ATRA therapy in the treatment of chronic ITP in adult patients and explore the possible mechanisms.

2. Material and methods

2.1. Patients

The study included 35 adults with chronic ITP who received ATRA therapy, who were enrolled in a clinical trial between January 2011 and August 2012 (trial NCT01668615 registered at http://www.clinicaltrials.gov). The study was approved by the institutional review boards of the First Affiliated Hospital of Soochow University, and written informed consent was obtained from all the participants in accordance with the Declaration of Helsinki. ITP diagnosis was according to criteria published in the guideline of American Society of Hematology [12]. Patients in whom treatment with standard dose corticosteroids and/or splenectomy fails, and who require further therapy because of unsafe platelet counts or clinical bleeding were included in this study. ATRA (10 mg, three times daily; Shandong Liangfu Group Pharmaceutical Co. Ltd. China) together

☆ *Grant support*: This work was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (YX21100214).

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http://dx.doi.org/10.1016/j.thromres.2016.02.013
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Table 1
Sequence of primers used in reverse transcription-polymerase chain reaction (RT-PCR).

Primer name	Forward primer (5′–3′)	Reverse primer (5′–3′)
T-bet	ACTGGAGCACAATCATCTGGG	TTGGGTGCAGTGTGGAAAGGC
GATA-3	GAAGAGTCCGGAGCTGTAC	AGGACGAGAAAGAGTGCC
Foxp3	GTGGCATCATCCGACAAGG	TGTGGAGGAACTCTGGGAAT
ROR-γ	GCTGGTTAGGATGTGCCG	GGATGCTTTGGCGATGA
GAPDH	GGAAGGTGAAGGTCGGAGTC	CGTTCTCAGCCTTGACGGT

Table 2
Summary of patient demographics and baseline characteristics.

	Responder (n = 19)	Non-responder (n = 16)	*p* value
Median (years; range)	40 (25–48)	39 (26–50)	NS
Sex (n, %)			
Male	8 (42)	7 (44)	NS
Female	11 (58)	9 (56)	NS
Platelet count ($\times 10^9$/ml; mean ± SD)			
Day 0	34 ± 13	35 ± 12	NS
Day 30	106 ± 29	36 ± 15	<0.05
Day 180	104 ± 20	35 ± 18	<0.05
Patients with any bleeding (n, %)			NS
Before	10 (53)	9 (53)	
During the treatment	4 (21)	4 (24)	
Cumulative bleeding events during treatment (n, %)	11	30	<0.05
Oral	5 (45)	15 (50)	
Cutaneous	4 (37)	10 (33)	
Genitourinary	2 (18)	5 (17)	
Intracranial	0 (0)	0 (0)	
Mean duration of disease (month; range)	26 (12–120)	24 (14–118)	NS
Splenectomy status (n, %)			
Yes	2 (11)	1 (7)	
No	17 (89)	15 (93)	NS
Immunosuppressant (n, %)			
Yes	9 (47)	10 (60)	NS
No	10 (53)	6 (40)	NS

NS: not significant.

with prednisone (10 mg, twice daily; Sinepharm Co. Ltd., Shanghai, China) was administered after discontinuation of previous treatment. The mean treatment duration was 3 months (range: 2–6 months). Peripheral blood were collected from all the patients prior to and 1 month after the treatment.

2.2. Response criteria

Patients were evaluated for response if they completed 4 week infusion. Respondents were classified as complete responders (CR), partial responders (PR), and nonresponders (NR). CR was defined as platelet count maintained ≥100×10^9/L for at least 3 months. PR was defined as a platelet >50×10^9/L after treatment or doubling of baseline. NR was defined as platelet count <50×10^9/L.

2.3. Antibodies and reagents

The following monoclonal antibodies used were from Beckman Coulter (Fullerton, CA): anti-CD4 conjugated to peridin chlorophyll protein (PerCP); anti-CD127 conjugated to phycoerythrin (PE); anti-CD25 conjugated to fluorescein isothiocyanate (FITC); anti-IL-4 FITC; anti-IFN-γ PE; isotype-matched, directly conjugated (FITC, PE and PerCP) control antibodies. Anti-IL-17 PE and related control antibody were purchased from BD Bioscience (San Diego, CA, USA).

2.4. Th1/Th2/Th17/Treg detection

Peripheral blood monocytes (PBMCs) in RPMI 1640 were stimulated with 25 ng/ml phorbol 12-myristate 13-acetate, 1 μg/ml ionomycin, and 10 μg/ml Brefeldin A (Sigma, St Louis, MO, USA) at 37 °C for 4 h. After incubation, PBMCs were washed with PBS twice. Then, the cells were stained with anti-CD4 at room temperature (RT) in dark for 15 min. After treatment with permeabilizing solution, cells were stained with anti-IL-17, anti-IL-4 and anti-IFN-γ at RT for 45 min. Moreover, in some settings of experiments, the cells were also stained with anti-CD25 and anti-CD127 at RT for 45 min without permeabilizing treatment. For each tube, at least 10,000 events were collected in a gate created around the viable lymphocyte population. Quadrants were applied to the isotype control dot plots to exclude nonspecific staining. The percentage of $CD4^+$ IL-17^+(Th17), $CD4^+$ IL-4^+(Th2), $CD4^+$ IFN-γ^+(Th1) and $CD4^+CD25^{high}CD127^{intensity/dim}$ (Treg) cell were determined.

2.5. Blood sampling and inflammatory cytokine detection

Peripheral blood was collected from median cubital vein into vaccutainer tubes containing ethylenediaminetetraacetic acid (EDTA)-anticoagulant. Serum was obtained by 3000 rpm centrifugation for 10 min and was stored in −80 °C before further processing.

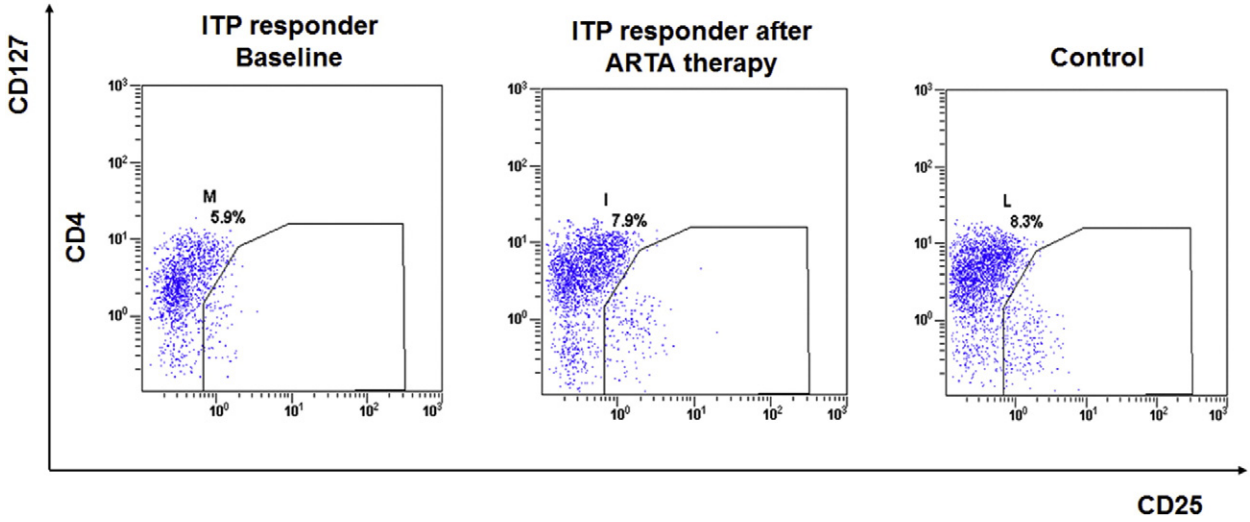

Fig. 1. *Representative dot-plots of regulatory T cell (Treg) cells as evaluated by flow cytometry*. Samples from the same patient, in this case a responder, were analyzed before and after all-*trans*-retinoic acid (ATRA) therapy. The sample of a control subject was employed for comparison. $CD4^+$ cells were sorted into Treg cells according to the expression of both CD25 and CD127.

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