Human Immunology 73 (2012) 240-247

Contents lists available at SciVerse ScienceDirect

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

Elevated interleukin-27 enhances the polarization of Th1/Tc1 cells and the production of proinflammatory cytokines in primary immune thrombocytopenia

Hui Yuan Li [a], Dong Lei Zhang [a], Jing Ge [a], Hu Zhou [a], Ai ping Qi [a], Li Ma [a], Feng Xue [a], Ze Ping Zhou [a,b], Ren Chi Yang [a,*]

[a] State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, People's Republic of China
[b] Department of Hematology, Second Affiliated Hospital of Kunming Medical University, Kunming 650101, People's Republic of China

ARTICLE INFO

Article history:
Received 1 September 2011
Accepted 6 December 2011
Available online 11 December 2011

Keywords:
Primary immune thrombocytopenia
Interleukin-27
Proinflammatory cytokines
Th1
Tc1

ABSTRACT

Primary immune thrombocytopenia (ITP) is an acquired, organ-specific, autoimmune disease with many immune dysfunctions. Interleukin-27 (IL-27) can regulate T cell differentiation. However, it is unclear whether IL-27 correlates with the dysfunctions of T cell differentiation in ITP patients. Thus, to determine the roles of IL-27 in ITP, we studied the expression of IL-27/IL-27 receptor in ITP patients. The results indicated that the levels of IL-27 in the plasma of untreated active ITP patients were higher than in normal controls. We next evaluated the contribution of IL-27 to T cell differentiation. Our results indicated that IL-27 increased T-bet expression, inhibited GATA-3 and ROR-γt expression, and promoted the secretion of tumor necrosis factor-α, interferon-γ, and granzyme B of peripheral blood mononuclear cells from ITP patients. Also, we confirmed that IL-27 induced the differentiation of T helper (Th)-1 and Tc1 cells. In conclusion, IL-27 might play an important role in the pathogenesis of ITP by inducing the polarization of Th1/Tc1 cells and the production of proinflammatory cytokines.

1. Introduction

Primary immune thrombocytopenia (ITP) is an acquired, organ-specific, autoimmune disease characterized by decreased platelet count resulting from increased platelet destruction and insufficient platelet production [1]. The etiology of ITP is complicated. Previous research has demonstrated that loss of tolerance is important in the pathogenesis of ITP [2,3]. Despite major advances regarding our understanding of the pathogenesis of ITP, the trigger that initiates the autoimmune process remains unknown. To date, the most widely accepted viewpoint is that more than 1 mechanism contributes to this disease: (1) Autoreactive B lymphocytes secrete antiplatelet antibodies, which lead to platelet destruction, T cell activation, and impaired maturation of megakaryocytes [4]. (2) The T cell subsets are skewed to T1 polarization in ITP, and the T helper (Th)-1:Th2 ratio is closely related to the etiology and disease status of chronic ITP [5–8]. (3) T cell–mediated platelet lysis may also contribute to platelet destruction. This expanded role of cytotoxic T cells may explain why a percentage of patients without measurable antiplatelet antibodies exists and again points to the heterogeneity of this disease [9]. (4) Reduced numbers and dysfunction of circulating regulatory T cells (Tregs) were observed in patients with chronic ITP [10–12]. In addition, inducible Tregs could mediate their tolerance-inducing effects on Th cells by modulating the antigen presentation function of dendritic cells (DCs), which suggested that a breakdown in tolerance induction may be the primary abnormality responsible for ITP induction [13]. (5) In addition, the dysfunction of antigen-presenting cells, natural killer (NK) cells, complements, and other immune elements was also involved in ITP [14–16].

Interleukin (IL)-27, a novel member of the IL-6/IL-12 family, is a heterodimeric cytokine that consists of Epstein–Barr-induced protein 3 (EBI3) and a p28 subunit [17]. IL-27 is produced by antigen-presenting cells when they activate upon stimulation through Toll-like receptor signaling [18,19]. The IL-27 receptor (IL-27R) complex consists of a specific IL-27Rα (WSX-1 or TCCR) subunit, which is unique for the binding of IL-27, and a signaling chain, gp130, which is shared with the receptor for IL-6. Both IL-27R chains have been observed to be expressed on several human cells, including T cells, NK cells, monocytes, mast cells, B cells, and DCs [20], which may explain the pleiotropic role of IL-27.

More importantly, several researchers have reported that IL-27 plays a variety of critical roles in the immune system. IL-27 can promote naive T cell proliferation [17], Th1 immune responses, and commitment of type 1 Tregs [21], but suppresses the expansion of Th17 and FoxP3$^+$ Tregs [22,23]. IL-27 also can augment the generation of cytotoxic T lymphocytes (CTL) with enhanced expression of granzyme B [24]. Based on these pleiotropic roles of IL-27 in immu-

* Corresponding author.
E-mail address: rcyang65@yahoo.com (R. Yang).

0198-8859/$36.00
doi:10.1016/j.humimm.2011.12.010

nity regulation, it also plays an important role in autoimmune disease and has potential therapeutic utility in complex immune diseases.

Therefore, to evaluate the role of IL-27 in the pathogenesis of ITP, we researched the expression level of plasma IL-27 and the *in vitro* activating effects of IL-27 on peripheral blood mononuclear cells (PBMCs) from ITP patients.

2. Subjects and methods

2.1. Patients and controls

Fifty-nine ITP patients were enrolled in this study. The diagnosis of ITP was based on criteria reported recently [25]. Informed consent was obtained from the patients' legal guardians or the patients themselves, and the study was approved by the hospital-based ethics committee.

Among the enrolled patients, 45 patients had active ITP, and 14 patients undergoing glucocorticoid or splenectomy treatment had a normal platelet count($>100 \times 10^9$). In addition, we divided the active patients into 2 subgroups, untreated and treated, based on whether they had received any glucocorticoid and/or other immunosuppressive treatments at least 1 month before sampling. The main features of the enrolled patients are presented in Tables 1, 2, and 3. A total of 25 age- and sex-matched healthy donors were enrolled as controls (median age 40 years; 10 men and 15 women).

Table 1
Clinical characteristics of active immune thrombocytopenia patients

Patients	Gender	Age (years)	Duration of disease (months)	Platelet count ($\times 10^9$/L)	Primary therapy
1	F	87	0.3	4	GC
2	F	65	1	9	GC
3	M	30	1	29	GC, DNZ
4	M	40	2	5	GC
5	F	36	2	18	GC
6	M	22	2	26	GC
7	M	28	2	39	GC IVIg
8	F	23	3	23	GC
9	F	48	3	60	GC
10	F	56	4	3	GC
11	M	36	4	15	GC, DNZ, IVIg
12	M	35	6	3	GC
13	M	65	7	25	GC, DNZ
14	F	27	10	13	GC
15	F	54	36	16	GC
16	F	50	60	54	GC, DNZ
17	M	81	60	75	GC
18	M	63	96	3	GC
19	M	22	98	22	GC
20	F	37	204	5	GC, IVIg
21	F	44	0.1	48	None
22	F	55	0.8	20	None
23	F	44	1	8	None
24	M	29	1	10	None
25	F	28	1	19	None
26	F	61	2	1	None
27	M	19	2	52	None
28	F	33	10	28	None
29	M	54	12	32	None
30	F	18	12	46	None
31	F	50	24	16	None
32	F	54	36	16	None
33	M	40	48	56	None
34	F	46	54	17	None
35	F	41	60	1	None
36	F	36	84	30	None
37	F	20	204	30	None
38	F	44	444	17	None
Median		40.5	6.5	18.5	
Range		18–87	0.1–444	1–75	

GC, glucocorticoid; IVIg, intravenous immunoglobulin; DNZ, danazol.

Table 2
Clinical characteristics of immune thrombocytopenia patients in remission

Patients	Gender	Age (years)	Duration of disease (months)	Platelet count ($\times 10^9$/L)	Primary therapy
1	F	18	12	128	SP
2	F	55	6	210	GC, IVIg, DNZ
3	M	50	36	100	GC
4	M	21	144	115	GC, IVIg
5	M	56	84	113	GC
6	F	69	60	103	GC
7	F	16	6	102	GC, IVIg
8	F	15	1	107	GC
9	F	36	6	129	GC
10	F	58	10	279	GC, IVIg
11	M	53	4	197	GC, IVIg
12	F	16	12	250	GC
13	F	31	3	117	GC
14	M	14	1	373	GC
Median		33.5	8	122.5	
Range		14–69	1–144	128–373	

GC, glucocorticoid; IVIg, intravenous immunoglobulin; DNZ, danazol; SP, splenectomy.

2.2. Preparation of plasma and PBMCs

Peripheral blood was collected into vaccutainer tubes containing EDTA-anticoagulant. Samples were centrifuged and plasma was collected and stored at −80°C until analysis. PBMCs were isolated from blood samples using Ficoll–Hypaque (1.077 g/mL) density gradient centrifugation.

2.3. Cell culture

Aliquots of 1×10^6 isolated PBMCs were cultured in 1 mL complete RPMI 1640 medium (containing 10% fetal bovine serum and 2 mmol/L glutamine/penicillin/streptomycin) stimulated with or without 50 ng/mL IL-27 (R&D Systems, Minneapolis, MN) for 48 hours at 37°C under 5% CO_2. After incubation, the cell culture supernatants were stored for detection of cytokines, and the cells were collected for extraction of total RNA.

2.4. Extraction of total RNA and reverse transcription of mRNA

Total RNA of the peripheral blood cells (1×10^6) was extracted using Trizol reagent (Invitrogen, Carlsbad, CA). The reverse transcription reactions were carried out using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) following the manufacturer's procedure.

2.5. Real-time polymerase chain reaction (PCR)

Real-time PCR was performed with 2× SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) using the Applied Biosystems Gene Amp 7500 sequence detection system. The primer sequences are listed in Table 4. For PCR amplification, an initial denaturation at 94°C for 10 minutes was followed by 40 cycles at 94°C for 15 seconds and 60°C for 1 minute. After PCR, a

Table 3
Clinical characteristics of active immune thrombocytopenia patients used for stimulation experiments

Patients	Gender	Age (years)	Duration of disease (months)	Platelet count ($\times 10^9$/L)	Primary therapy
S1	F	14	2	41	GC
S2	F	34	24	10	GC
S3	M	71	36	2	GC, IVIg
S4	F	17	2	2	None
S5	M	44	1	3	None
S6	F	22	11	5	None
S7	F	32	240	1	GC

GC, glucocorticoid; IVIg, intravenous immunoglobulin.