International Immunopharmacology 55 (2018) 63–68

Contents lists available at ScienceDirect

International Immunopharmacology

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

Increased ADAM10 expression in patients with immune thrombocytopenia

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ARTICLE INFO

Keywords:
Immune thrombocytopenia
ADAM10
TIMP3
FasL
LAG-3

ABSTRACT

Immune thrombocytopenia (ITP) is an autoimmune disease, which is characterized by abnormal of T immunity. A disintegrin and metalloproteinase (ADAM) 10, a member of proteinase family, has been demonstrated to regulate T cell proliferation and effector function. Considering the closely association of dysregulation of T cell function with ITP, whether ADAM10 involves in the pathogenesis of ITP remains unclear. In this study, 54 active ITP patients, 18 ITP in remission and 24 age and gender matched healthy control were enrolled. Peripheral blood mononuclear cells (PBMCs) were isolated from patients and control for isolation of RNA and plasma which were used to measure mRNA level of ADAM10 and tissue inhibitor of metalloproteinase 3 (TIMP3) by quantitative real-time PCR and soluble level of FasL and lymphocyte activation gene-3 (LAG-3) in plasma by ELISA. Meanwhile, T cell activation was measured by flow cytometry. Our results showed significantly higher expression of ADAM10 and lower expression of TIMP3 in active ITP patients compared with control, which were all restored into normal level in remission patients. Consistent with the expression profile of ADAM10, increased soluble plasma level of FasL and LAG-3 were observed in active ITP patients and reduced to normal level in patients in remission. Furthermore, increased T cell activation as demonstrated by higher expression of HLA-DR and CD69 were found in active ITP patients. In conclusion, elevated expression of ADAM10 was associated with the pathogenesis and development of ITP and therapeutically targeting it might be a novel approach for the treatment of ITP.

1. Introduction

Immune thrombocytopenia (ITP) is a heterogeneous autoimmune disease, characterized by antiplatelet autoantibodies mediated accelerated platelet destruction and impaired platelet production, leading to reduced platelet count (thrombocytopenia), rendering patients a higher risk of bleeding [1,2]. The pathophysiology of ITP is becoming more and more complicated, with lots of factors involved. The pathogenesis of ITP is believed to be mainly resulted from enhanced Fc receptor (FcR)-mediated phagocytosis of autoantibodies-opsonized platelets, leading to destruction by macrophages in the reticuloendothelial system in the spleen [3].

Apart from antiplatelet autoantibodies, cellular immune dysregulation has also been shown to be involved in the pathogenesis of ITP [3]. Given the critical role in the regulation of immune response and attack, T cells have been reported to play an important role in the development of ITP [4,5], as demonstrated by imbalanced T helper (Th) 1 and Th2 cells [6], less apoptotic and more clonal expansion or proliferation of platelet autoreactive T cells in ITP patients, resulting in an imbalanced secretion of inflammatory cytokines and subsequent reduced expression of regulatory T cells (Tregs), which play a critical role in controlling appropriate immune response and autoreactivity [7,8]. In

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https://doi.org/10.1016/j.intimp.2017.12.004
Received 18 September 2017; Received in revised form 21 November 2017; Accepted 5 December 2017
Available online 22 December 2017

addition, increased secretion of cytokines can influence the interaction between B and T lymphocytes leading to proliferation of pre-existing B cells and subsequent production of high affinity autoantibodies in patients with ITP [9]. Apart from T cells, dysregulation of B cells have also been associated with ITP as demonstrated by increased levels of circulating B cells excreting antiplatelet autoantibodies and elevated serum level of B cell-activating factor in patients with ITP [10–12].

A disintegrin and metalloproteinase (ADAM) 10 is a member of proteinase family, consisting of an N-terminal signal sequence, a prodomain, a highly conserved metalloproteinase domain, a disintegrin domain with a cysteine-rich region, a transmembrane domain and a cytoplasmic tail with binding affinity to SH3 domains [13,14]. ADAM10 is synthesized as an inactive zymogene, in which the latency is maintained by their autoinhibitory pro-domains via the “cysteine switch” mechanism [15]. A proteolytic processing then transforms ADAM10 into an active form through removal of the prodomain from the precursor protein in the trans-Golgi network. Once activated, ADAM10 can execute ectodomain shedding as well as regulates intramembrane proteolysis of transmembrane proteins, including amyloid plaque precursor, cadherins, chemokines, Notch receptors, LAG-3, FasL and CD23 [16]. Proteolytic processing of these substrates is demonstrated to be involved in the pathogenesis of several diseases, such as cancer, inflammation or autoimmunity [16–18], indicating ADAM10 might participate in the development or occurrence of diseases. In addition, a previous study demonstrated that ADAM10 regulates T cell proliferation and effector function through proteolytic processing of lymphocyte activation gene-3 (LAG-3) [19], a novel inhibitory molecule that is required for maximal T cell function as well as controls T cell expansion and hemostasis [20–22], suggesting ADAM10 might play a role in the regulation of T cell activation and function. Furthermore, ADAM10 is showed to regulate FasL cell surface expression in human T cells through ectodomain shedding of the apoptosis-inducing Fas ligand (FasL), modulate FasL-induced T cell cytotoxicity as well as activation-induced cell death [23], further supporting the role of ADAM10 in T cell function.

As abnormal T cell function has been demonstrated to play a critical role in the development or pathogenesis of ITP and ADAM10 is involved in the regulation of T cell activation or function, whether ADAM10 is involved in the development of ITP remains poorly understood. In the present study, we aimed to evaluate the expression profile of ADAM10 in patients with active ITP as well as ITP patients with remission.

2. Materials and methods

2.1. Patients

From March 2015 to December 2016, 54 primary ITP patients (22 males and 32 females with a median age of 45, ranged from 25 to 70 years old) (Table 1) from Department of Hematology, the Affiliated Hospital of Xuzhou Medical University, Xuzhou China were recruited into this study. ITP was diagnosed based on the criteria from international working group [24]. These 54 primary ITP patients with a platelet count $< 50 \times 10^9$/l ranging from 3 to 49 $\times 10^9$/l were defined as active ITP (Table 1). Patients with diabetes, cardiovascular diseases, hypertension, active or chronic infection were excluded. Meanwhile, 18 ITP patients (8 males and 10 females with a median age of 42 ranging from 18 to 75 years old) (Table 1) achieved remission after treated with prednisolone, intravenous immunoglobulin or glucocorticoid, including complete response and response after treatment, with the response criteria being defined by the consensus report of international working group [24]. ITP patients in remission had a median platelet count of 120×10^9/l ranged from 65 to 190 $\times 10^9$/l. 24 age and gender matched healthy individuals, consisted of 10 males and 14 females with a median age of 43 ranged from 20 to 72 years old, were included as a control (Table 1). The platelet counts in healthy individuals ranged from 150 to 320 $\times 10^9$/l with a median count of 245 $\times 10^9$/l. Ethical approval for this study was obtained from the Medical Ethics Committee of the Affiliated Hospital of Xuzhou Medical University, Xuzhou China. Informed consent was obtained from all participants before enrollment in the study.

Table 1
Clinical characteristics of ITP patients and control.

	Active ITP	ITP in remission	Control
N	54	18	24
Male/female	22/32	8/10	10/14
Age (range)	45 (25–70)	42 (18–75)	43 (20–72)
Platelet count (range) ($\times 10^9$/l)	21 (3–49)	120 (65–190)	245 (150–320)

2.2. Plasma preparation

Venous whole blood was collected from patients or control into tubes with EDTA as anti-coagulant followed by centrifuging anti-coagulant blood at 2000 rpm for 20 min to obtain plasma. Isolated plasma was stored at − 80 °C until further analysis.

2.3. Isolation of peripheral blood mononuclear cells and RNA extraction

Peripheral blood mononuclear cells (PBMCs) were isolated from collected venous blood through gradient centrifugation of anti-coagulated blood on Ficoll-Paque Plus (Sinopharm Chemical Reagent Ltd., China) at 2000 rpm for 20 min.

RNA was extracted from PBMCs using TRIZOL reagent (Life Technology, Carlsbad, CA, USA) according to the manufacturer's instruction. The quantitation and quality of isolated RNA was evaluated by measuring the absorbance of RNA at 260 nm on a spectrophotometer and agarose gels, respectively.

2.4. Measurement of ADAM10 and TIMP3 mRNA level by quantitative real-time PCR

Total RNA was reversely transcripted into cDNA using cDNA synthesis kit (ThermoFisher Scientific, Waltham, MA, USA) which was used for quantified measurement of mRNA expression of ADAM10 and TIMP3 by real-time PCR with GAPDH as internal control as previously described [25–27]. Amplification was performed in triplicate on LightCycler® R480 II (Roche Life Science) in a total volume of 20 μl, consisting of 10 μl SYBR Green qPCR Super Mix, 0.5 μl forward primer (10 μM), 0.5 μl reverse primer (10 μM), 5 μl cDNA and 4 μl sterile water. Primers for ADAM10, TIMP3 and GAPDH were listed in Table 2. The PCR reaction conditions were as follows: Initial denaturation (95 °C for 5 min), 40 cycles of denaturation (95 °C for 20 s); annealing (60 °C for 15 s) and extension (72 °C for 15 s). The melting curve analysis of the amplification products was performed at the end of each PCR reaction. The relative mRNA expression of target genes was calculated by comparative Ct method which is performed by using the following formula: relative expression = $2^{-\Delta\Delta}$Ct.

Table 2
Primers for quantitative real-time PCR.

Gene	Primer sequence (5′-3′)	Length (bp)
ADAM10	F: GGATTGTGGCTCATTGGTGGGCA R: ACTCTCTCGGGGCCGCTGAC	203
TIMP3	F: TGATGGCAAGATGTACACGG R: GAAGTCACAAAGCAAGGCAG	150
GAPDH	F: TGAAGGTCGGAGTCAACGGATT R: CCTGGAAGATGGTGATGGGATT	225