Human Immunology 77 (2016) 944–951

Contents lists available at ScienceDirect

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

Involvement of genes encoding apoptosis regulatory factors (*FAS*, *FASL*, *TRAIL*, *BCL2*, *TNFR1* and *TNFR2*) in the pathogenesis of autoimmune thyroid diseases

Naoya Inoue [a,b], Mikio Watanabe [a], Naoko Ishido [a], Arisa Kodu [a], Hayato Maruoka [a], Yuka Katsumata [a], Yoh Hidaka [b], Yoshinori Iwatani [a,*]

[a] Department of Biomedical Informatics, Division of Health Sciences, Osaka University, Graduate School of Medicine, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Laboratory for Clinical Investigation, Osaka University Hospital, 2-15 Yamadaoka, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 12 November 2015
Revised 27 June 2016
Accepted 21 July 2016
Available online 22 July 2016

Keywords:
Apoptosis
Autoimmune thyroid disease
Polymorphism
Intractability
Severity

ABSTRACT

Apoptosis is necessary for the maintenance of self-tolerance by eliminating autoreactive immune cells in the periphery. To clarify the association between the pathogenesis of autoimmune thyroid disease (AITD) and genes encoding apoptosis regulatory factors, we genotyped the *FAS* −1377G/A, −670A/G, *FASL* −844C/T, *TRAIL* −716C/T, *BCL2* −938C/A, +127G/A, *TNFR1* −383A/C and *TNFR2* +676T/G polymorphisms. The frequencies of the *FASL* −844CC and *BCL2* −938AA genotypes were significantly lower in AITD patients than in control subjects ($P = 0.0101$ and 0.0307, respectively). The frequency of the *TNFR2* +676TT genotype was significantly lower in Graves' disease (GD) patients than in controls ($P = 0.0284$). The serum sFasL level was significantly higher in GD and Hashimoto's disease (HD) patients than in control subjects ($P = 0.0003$ and 0.0017, respectively). The serum sFasL levels in control subjects were significantly lower than those in intractable GD, GD in remission, and HD without treatment ($P = 0.0310$, 0.0007 and 0.0002, respectively). The serum sFasL levels in HD with treatment were significantly lower than those in HD without treatment ($P = 0.0490$). The polymorphisms in genes encoding apoptosis regulatory factors (*FASL, BCL2*) and serum levels of sFasL may be associated with immune dysregulation.

1. Introduction

Autoimmune thyroid disease (AITD), such as Hashimoto's disease (HD) and Graves' disease (GD), are archetypes of organ-specific autoimmune disease [1,2]. The severity of HD and intractability of GD vary among patients and are highly difficult to predict. A portion of patients develop hypothyroidism early in life, and a portion maintain a euthyroid state in old age, despite the passage of time. Certain patients with GD achieve remission through medical treatment, while others do not. We have previously reported an association between the genetic producibility of immune mediate factors and prognosis of AITD [3–9].

Immunological self-tolerance is maintained by multiple mechanisms in the immune system [10–12]. Apoptosis is a necessary mechanism for the maintenance of self-tolerance by eliminating autoreactive immune cells in the periphery [13–15]. Failure of this system involves the induction and exacerbation of autoimmune diseases [13–15]. The Fas-Fas ligand (FasL) interaction is one of the primary pathways for apoptosis, which is involved in the pathogenesis of HD [16]. We previously reported that the intensity of Fas expression on $CD4^+$ and $CD8^+$ cells increased in intractable GD and severe HD patients [17], and intrathyroidal regulatory T cells were decreased by apoptosis in AITD patients [18].

Although the function of soluble FasL (sFasL) in apoptosis by FAS-FASL is unclear, soluble Fas (sFas) and sFasL levels are increased in AITD patients [19–22] and may be useful for predicting the prognosis. Individuals with the AA genotype of the −670A/G polymorphism (rs1800682) in the *FAS* gene show a significantly higher level of serum sFas than those with the GG genotype [23]. Moreover, the higher SP-1 binding ability of the G allele of the *FAS* −1377G/A polymorphism (rs2234767) suggested the effect of this polymorphism on the expression of Fas and production of sFas [24,25]. Conversely, the promoter activity of the C allele of the *FASL* −844C/T polymorphism (rs763110) is significantly higher than that of the T allele, and the expression of FasL in individuals with

* Corresponding author.
E-mail address: iwatani@sahs.med.osaka-u.ac.jp (Y. Iwatani).

http://dx.doi.org/10.1016/j.humimm.2016.07.232
0198-8859/

the *FASL* −844CC genotype is significantly higher than that those with the −844TT genotype [26].

Other factors that regulate apoptosis include tumor necrosis factor-related apoptosis inducing ligand (TRAIL), B cell lymphoma-2 (Bcl-2) and tumor necrosis factor receptor (TNFR). TRAIL activates intercellular apoptotic responses in various cells [27], induces a milder form of experimental autoimmune thyroiditis by suppressing the Th1 response [28] and promotes the differentiation of regulatory T (Treg) cells [29]. The promoter activity of the C allele in the *TRAIL* −716C/T polymorphism (rs12488654) is significantly higher than that of the T allele [30].

Bcl-2 is an anti-apoptosis factor, and high expression of Bcl-2 promotes cell survival [31]. The C allele of the −938C/A polymorphism (rs2279115) is correlated with lower Bcl-2 expression than the A allele [32–34]. Moreover, the antiapoptotic function of the A allele of the Ala43Thr (+127G/A) polymorphism (rs1800477) is lower than that of the G allele [35,36], and the frequency of the A allele is significantly lower in autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [35].

We previously reported that higher genetic TNF-α producibility was associated with the development of AITDs and activity of GD [37]. TNF-α binds to two TNFR types, TNFR1 and TNFR2 [38,39]. There is a death domain in TNFR1, but not in TNFR2, that induces apoptosis [40]. TNFR2 can activate NF-κB, which inhibits TNF-α-induced cell death and protects cells from human neuronal cell from injury [41–43]. The frequency of the −383C allele of the *TNFR1* −383A/C polymorphism (rs2234649), which is correlated with higher promoter activity, is higher in type 1 diabetes patients than in controls [44]. The soluble TNFR levels released by T cells are lower in individuals with the +676G allele of the *TNFR2* +676T/G (M196R) polymorphism (rs1061622) than in individuals with the T allele [45].

In this study, we genotyped the functional polymorphisms in the *FAS*, *FASL*, *TRAIL*, *BCL2*, *TNFR1* and *TNFR2* genes and examined the sFas and sFasL levels to clarify the associations between these apoptosis regulatory factors and the pathogenesis of AITD.

2. Materials and methods

2.1. Subjects

Among 123 HD patients, we screened for the *FAS*, *FASL*, *TRAIL*, *BCL2*, *TNFR1* and *TNFR2* polymorphisms in 57 patients with HD who developed moderate to severe hypothyroidism before the age of 50 years and were treated daily with thyroxine (severe HD) and in 43 untreated euthyroid patients with HD who were older than 50 years of age (mild HD). All of the patients with HD were positive for anti-thyroid microsomal antibody (McAb) and/or anti-thyroglobulin antibody (TgAb), and all of the patients with mild HD had palpable diffuse goiters.

In 160 GD patients, we also examined 75 euthyroid patients with GD who had been treated with methimazole for at least 5 years and were still positive for anti-thyrotropin receptor antibody (TRAb) (intractable GD), 48 patients with GD in remission who had maintained a euthyroid state and were negative for TRAb for more than 2 years without medication (GD in remission) and 87 healthy volunteers who were euthyroid and negative for thyroid autoantibodies. All of the patients and control subjects were Japanese. Written informed consent was obtained from all of the subjects, and the study protocol was approved by the Ethics Committee of Osaka University. Genomic DNA was isolated from EDTA-treated peripheral blood mononuclear cells using a commercially available kit (QIAamp DNA Blood Mini Kit; Qiagen, Tokyo, Japan).

Table 1
The primer sequences, PCR conditions and restriction enzymes in PCR-RFLP method.

Gene	Polymorphism	Primer sequences		PCR conditions	Restriction enzymes
		Forward primer	Reverse primer		
FAS	−1377G/A (rs2234767)	5′-TGTGTGCACAAGGCTGGCGC-3′	5′-TGCATCTGTCACTGCACTTACCACCA-3′	95 °C for 5 min, (95 °C for 30 s, 68 °C for 30 s, 72 °C for 30 s) × 35cycles, 72 °C for 7 min	BstUI
	−670A/G (rs1800682)	5′-CTACCTAAGAGCTATCTACCGTTC-3′	5′-GGCTGTCCATGTTGTGGCTGC-3′	95 °C for 5 min, (95 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s) × 35cycles, 72 °C for 7 min	BstNI
FASL	−844C/T (rs763110)	5′-CAGCTACTCGGAGGCCAAG-3′	5′-ATTTACCCTGACCTGCCGATCAC-3′	95 °C for 5 min, (95 °C for 30 s, 68 °C for 20 s, 72 °C for 30 s) × 35cycles, 72 °C for 7 min	BsrDI
TRAIL	−716C/T (rs12488654)	5′-CATGCCTGTGTGTTAGGCTGGAGA-3′	5′-GCTGGGTCCTGAATCTGAGGGTAA-3′	95 °C for 5 min, (95 °C for 30 s, 54 °C for 30 s,t 72 °C for 30 s) × 35cycles, 72 °C for 7 min	MnlI
BCL2	−938C/A (rs2279115)	5′-CCCGGCTCCTTCATCGTCCC-3′	5′-CCGGTATTCGCAGAAGTCCTGT-3′	95 °C for 5 min, (95 °C for 30 s, 63 °C for 30 s,t 72 °C for 30 s) × 35cycles, 72 °C for 7 min	BccI
	+127G/A (rs1800477)	5′-CCCGTTGCTTTTCCTCTGGGA-3′	5′-GGGAGGAGAAGATGCCCGCCGCGGGG-3′	95 °C for 5 min, (95 °C for 30 s, 63 °C for 30 s, 72 °C for 30 s) × 35cycles, 72 °C for 7 min	BglI
TNFR1	−383A/C (rs2234649)	5′-TTATTGCCCCTTGGTGTTTGGTTG-3′	5′-GGGGAAGAGTGAGGCAGTGT-3′	95 °C for 5 min, (95 °C for 30 s, 61 °C for 30 s, 72 °C for 30 s) × 30cycles, 72 °C for 5 min	BglII
TNFR2	+676T/G (rs1061622)	5′-ACTCTGTCCCCTGCTGCCTC-3′	5′-GAGCGAAGTCGCCAGTGCTC-3′	96 °C for 5 min, (95 °C for 60 s, 62 °C for 30 s, 72 °C for 30 s) × 35cycles, 72 °C for 5 min	NlaIII