A novel mutation (T61I) in the gene encoding tumour necrosis factor receptor superfamily 1A (TNFRSF1A) in a Japanese patient with tumour necrosis factor receptor-associated periodic syndrome (TRAPS) associated with systemic lupus erythematosus


Objective. To identify potential mutations in the tumour necrosis factor receptor superfamily 1A gene (TNFRSF1A) in a Japanese female patient with recurrent fever complicated by systemic lupus erythematosus (SLE), and in her family members.

Methods. DNA sequencing of exons 1–10 of the TNFRSF1A gene was performed to determine mutations that might be associated with the tumour necrosis factor receptor-associated periodic syndrome (TRAPS). Moreover, the TNFRSF1A gene was examined in Japanese patients with autoimmune diseases, including SLE, rheumatoid arthritis (RA), mixed connective tissue disease (MCTD) and Behçet’s disease, and in healthy Japanese controls. Enzyme-amplified sensitivity immunoassay (EASIA) analysis was used to assess serum levels of TNF, the 55-kDa TNF receptor (TNFRSF1A) and the 75-kDa TNF receptor (TNFRSF1B). Membrane TNFRSF1A expression was analysed on the surface of peripheral blood mononuclear cells by flow cytometry.

Results. A novel mutation, a heterozygous C to T transition in exon 3 which substitutes an isoleucine for a threonine at position 61 (T61I) was detected in the TNFRSF1A gene derived from the genomic DNA of a Japanese female TRAPS patient. Two nieces and one nephew, all with a similar clinical phenotype, also possessed the same TNFRSF1A mutation. We further demonstrated the same mutation in five of 60 SLE patients (8.3%) and in five of 120 healthy individuals (4.2%), with no significant differences. Although high titres of serum TNF and soluble TNFRSF1B protein were observed in this patient, low titres of soluble TNFRSF1A protein were detected. However, a defect in TNFRSF1A shedding in vitro was not observed in monocytes derived from this patient.

Conclusion. This is the first report of a TRAPS patient associated with SLE with a novel TNFRSF1A mutation (T61I).

Key words: TRAPS, SLE, Mutation, TNF receptor, TNFRSF1A, Hereditary periodic fever syndrome.
Patients and methods

A Japanese family with recurrent fever

A 27-yr-old Japanese female patient was diagnosed with SLE at the age of 21 yr based on butterfly rash, fever, arthralgia, proteinuria, anaemia, antinuclear antibodies, a high titre of anti-double-stranded DNA (anti-ds DNA) antibodies (142.0 IU/ml), and low serum level of complement (CH50, 14.9 CH50/ml; C3, 33.0 mg/dl). After 3 yr of steroid therapy, recurrent fever appeared at the end of September, 2000 (Fig. 1). At this time, inflammatory signs were markedly elevated (high serum level of CRP and leucocytosis). In contrast, signs related to SLE, including butterfly rash, proteinuria, anaemia, high titres of anti-ds DNA antibodies and low levels of complement, were not observed in this period. The patient had undergone extensive diagnostic evaluation for infections, malignant and rheumatic aetiologies of her symptoms, with no positive findings. This led us to consider another aetiology for her illness. The patient also developed myalgia, arthralgia, a ‘Still’s rash’ on the upper and lower extremities, and conjunctivitis during episodes of high fever. However, neither abdominal pain nor chest pain was present. Further examination demonstrated fasciitis in both thighs, detected by Ga scintogram, magnetic resonance imaging (MRI) (Fig. 2) [14, 23] and muscle and fascial biopsy (Fig. 3). CD68-positive cells were mainly infiltrated in fascia (data not shown), leading to the diagnosis of monocytic fasciitis [9, 24, 25]. We observed this patient carefully during more than 3 yr, and recurrent fever occurred repeatedly. Serum CRP and aldolase but not creatine phosphokinase (CPK) were elevated during periods of high fever (Fig. 1), suggesting that TRAPS symptoms resulted from the propagation of the fascial inflammation. Her family history demonstrated that two nieces (no. 11; 18 yr old; no. 17, 4 yr old) and 1 nephew (no. 14, 6 yr old) were also referred for recurrent fever (fever over 38°C lasting more than 7 days) with skin eruption (nos 11 and 17), arthralgia (no. 17) or abdominal pain (nos 11, 14 and 17), establishing the diagnosis of
TRAPS according to Hull’s criteria [12]. They did not fulfil the criteria for SLE. Although the patient was from Nagasaki, her parents were not present in Nagasaki at the time of the atomic bomb detonation.

Japanese patients with autoimmune disease

We studied 154 patients diagnosed as having SLE (57 females, three males), RA (54 females, eight males), MCTD (16 females), and Behçet’s disease (10 females, six males), as well as 120 healthy controls. All of the patients and controls were Japanese, and the patients were followed at our out-patient rheumatology facility at Nagasaki University Hospital.

Genomic DNA analysis

This study was approved by the ethics review board of Nagasaki University, and all participants gave their informed consent. Genomic DNA was prepared from peripheral blood. Polymerase chain reaction (PCR) amplification of each of the 10 TNFRSF1A-coding exons was performed using oligonucleotide primer pairs specific for exons 1–10 of the TNFRSF1A gene [14]. The sequences of the primers were as follows: 1A 5'-CAGCACTGCGCTGCCACAC-3', 1B 5'-AGGTGGCGCTCGG-3', 2A 5'-CCTCTCTTGATGGTGTCTCC-3', 2B 5'-GCAGCACCCCAGACCTGAGG-3', 3A 5'-GTGTTCTCACCAGCCGCT-3', 3B 5'-CCCACACACCACCTCAAGC-3', 4A 5'-GAGGATGCGAGGACTCATACC-3', 4B 5'-GG

Fig. 2. Magnetic resonance imaging of the thigh. Sagittal (A) and coronal (B) views of the middle thighs of a patient with the T61I mutation using STIR magnetic resonance imaging, demonstrating high-intensity signals in fascia (arrow).

Fig. 3. Pathological findings in monocytic fasciitis. Cross-section of the frozen skeletal muscle biopsy samples from the right thigh, showing small numbers of infiltrated cells around endothelium, with well-preserved, intact myofibres and endomysium (A, H&E stained; magnification ×200). In contrast, the adjacent fascia was markedly inflamed with cells (B, H&E staining; magnification ×200).
AAGTGGCCACCCGATGGG-3', 5A 5'-TACCTTCTCTTCTCTCTGCTGTGGG-3', 5B 5'-TGCCATGCTAATGTTCCATC-3', 5-7A 5'-CCTTTGACTGCTGCTTCTGGT-3', 6–7B 5'-CACCACATGTTCCTCTCACCC-3' (exon 6, intron 6 and exon 7 were amplified as a single product), 8A 5'-AGCTGAGTCCAGGTGACCAG-3', 8B 5'-GAAAGTGAAGGATGATTCCAGG-3', 9A 5'-CCCTCACC-3', 10% DMSO, 0.01% gelatine, 200 mM deoxy-NTPs, 1 mM of each primer, 0.25 U AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA, USA). The cycling conditions were as follows: genomic DNA (50–100 ng), PCR buffer containing 10 mM Tris–HCl (pH 9.2), 50 mM KCl, and 1 mM MgCl2, 10% DMSO, 0.01% gelatine, 200 mM deoxy-NTPs, 1 mM of each primer, 0.25 U AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA, USA). The cycling conditions were 5 min at 94°C followed by 32 cycles consisting of 45 s at 94°C, 30 s at 55°C and 45 s at 72°C. The PCR products were purified using a Microcon-100 filter (Amicon, Beverly, MA, USA) before both strands were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, PE Applied Biosystems). The reactions were analysed on an ABI Prism 3300 DNA Sequencer, as previously described [26].

Restriction analysis of the T61I mutation

Exon 3 of TNFRSF1A was PCR amplified in each member of the family, using primers 3A and 3B. The PCR products were digested by HphI (MBI Fermentas, Lithuania) restriction enzyme at 37°C for 1 h and fragments were separated in 2.5% agarose gels stained with ethidium bromide. The PCR products with the T61I mutation produce two discrete fragments (163 and 51 bp), whereas those with no T61I mutation produce three fragments (107, 56 and 51 bp).

HLA-A, B, C typing and HLA-DRB1, DQB1 typing

In our TRAPS patient, HLA typing for antigens of class I (HLA-A, B and C) was performed with the lymphocyte microcytotoxicity test [27]. DNA typing of the HLA class II gene (HLA-DRB1 and DQB1) was performed with the PCR-SSO (sequence-specific oligonucleotide) method [28, 29].

Measurement of soluble TNF receptors and TNF

Soluble TNFRSF1A (p55), soluble TNFRSF1B (p75) and TNF were measured in the serum using a solid-phase enzyme-amplified sensitivity immunoassay (EASIA) performed in microtiter plates according to the manufacturer's instructions (Biosource, Nivelles, Belgium), as previously described [30]. The assay is based on an oligonucleotide system in which a blend of monoclonal antibodies directed against distinct epitopes of soluble TNFRSF1A, soluble TNFRSF1B or TNF are employed, resulting in highly sensitive detection. We examined 33 serum samples from our TRAPS patient, and serum from a 16-yr-old male with SLE (before and after therapy), a 19-yr-old female with virus-associated haemophagocytic syndrome (VAHS) (before and after therapy), and 3 normal individuals. Each sample was examined in duplicate and data were calculated as the mean.

Flow cytometry histograms of TNFRSF1A expression in monocytes after PMA and ionomycin stimulation

Peripheral lymphocytes derived from a TRAPS patient and an SLE patient were purified by centrifugation over Ficoll–Hypaque. The lymphocytes were incubated with 10% fetal calf serum (FCS) RPMI in 5% CO2 at 37°C for 15 min, then phorbol 12-myristate 13-acetate (PMA) (final concentration 20 ng/ml; Sigma, St Louis, MO, USA) and ionomycin (final concentration 500 ng/ml; Sigma) were added to the media. After incubation for 10 or 60 min, lymphocytes were harvested and washed once with cold washing buffer [phosphate-buffered saline (PBS) containing 2% FCS and 0.1% azide sodium]. The dual immunofluorescence analysis method has been described in detail elsewhere [30]. Briefly, cell pellets were incubated with a fluorescein isothiocyanate (FITC)-conjugated anti-TNFRSF1A monoclonal antibody (mAb) (R&D, Minneapolis, MN, USA) and PCS-coupled anti-CD14 mAb (Beckman Coulter, Hialeah, FL, USA) or a FITC-conjugated anti-mouse IgG1 mAb (R&D) and PCS-coupled anti-CD14 mAb for 45 min at 4°C. After incubation, the cells were washed once with cold washing buffer. The dual-immunofluorescence experiments were analysed with a flow cytometer (Epics XL; Beckman Coulter).

Immunohistochemical analysis of tissues

Biopsy samples were fixed in 4% paraformaldehyde in PBS and were immersed successively in 10, 15 and 20% sucrose. Tissues were then frozen in liquid nitrogen and stored at −80°C until use. Tissue sections (4 mm thick) were cut and mounted on glass slides precoated with aminopropyltriethoxysilane. Dried cryostat sections of snap-frozen tissues were fixed with a mixture of methanol and acetone (vol/vol, 1/1) for 10 min on ice. Endogenous peroxidase activity was inhibited by immersing the section in 3% H2O2. After blocking with 10% mouse IgG for 10 min, sections were incubated with anti-CD68 mAbs (Dako, Carpinteria, CA, USA), anti-CD3 mAbs (Becton Dickinson, Mountain View, CA, USA) or anti-CD19 mAbs (Becton Dickinson) in PBS containing 1% fetal bovine serum (FBS) for 60 min in a humidified chamber at room temperature. These sections were washed with PBS containing 0.25% Brij and then stained using the streptavidin–biotin method (Nichirei, Tokyo, Japan) [31]. Haematoxylin and cosin (H&E) staining was performed using dried cryostat sections of snap-frozen tissues after fixation.

Statistical analysis

Data were analysed with Fisher's exact probability test. P < 0.05 was considered statistically significant.

Results

Mutation analysis of the TNFRSF1A gene and pedigree of a TRAPS family

Genomic DNA analysis identified a single mutation, a heterozygous C to T transition in exon 3, which substitutes an isoleucine for a threonine at position 61 (T61I) (Fig. 4A). No other mutations were observed in exons 1–10 of the TNFRSF1A gene using DNA sequencing analysis. The T61I mutation destroys an HphI restriction site determined by DNA strider 1.2. Two nieces and one nephew, all with a similar clinical phenotype, also possessed the same T61I mutation in the absence of TRAPS symptoms (Fig. 4B). This mutation was detected in another five family members (data not shown). Another five family members have the same mutation in the absence of TRAPS symptoms (Fig. 4B). This mutation was also confirmed by DNA sequencing analysis. As this patient (no. 8) also met diagnostic criteria for SLE, we examined whether the T61I polymorphisms was commonly associated with SLE. Surprisingly, this mutation was detected in another five SLE Japanese patients out of 60 analysed (8.3%), whereas no change of T61I was seen in other patients with RA, MCTD or Behçet's disease. As the same mutation was also found in five healthy Japanese individuals out of 120 (4.2%), we could not detect significant differences (P = 0.385) between SLE and healthy controls in the percentage of T61I mutation.

Statistical analysis

Data were analysed with Fisher’s exact probability test. P < 0.05 was considered statistically significant.
The arrow points to the heterozygous C → T transition, resulting in a threonine-to-isoleucine substitution in the upper panel. (B) Pedigree of the family. The T61I mutation (square or circle with a black point) was confirmed by DNA sequencing analysis. The genomic DNA from two family members (nos 12 and 13) could not be provided (NT). Shaded symbols indicate the existence of TRAPS symptoms (nos 8, 11, 14 and 17).

Expression of TNFRSF1A on monocytes from patients after PMA and ionomycin stimulation

As serum concentrations of the soluble form of TNFRSF1A are low, we assayed for defects in receptor shedding from monocytes. Peripheral lymphocytes derived from a TRAPS patient and a patient with SLE without the T61I mutation (control) were stimulated with PMA and ionomycin for 10 or 60 min, then stained with anti-TNFRSF1A mAb and anti-CD14 mAb. Figure 6 shows that normal shedding was observed in CD14-positive cells (monocytes) from both a TRAPS patient and a patient with SLE without T61I mutation, suggesting that abnormal shedding does not account for the low concentrations of the soluble form of the TNFRSF1A in sera from this TRAPS patient.

Discussion

A critical feature of this case was the presence of high fevers in the absence of SLE disease activity. During high fever (after the end of September 2000), inflammatory disease signs were increased, despite normal levels of serum complement, leading us to consider another aetiology for her recurrent fever (rather than SLE flare). Periodic high fever appeared in association with monocyctic fasciitis in both thighs, as determined by Ga scintigram and MRI (Fig. 2) [14, 23] and confirmed by immunohistological studies (CD68-positive cells were seen to infiltrate into fascia; data not shown) [9, 24, 25]. In spite of high titres of TNF and soluble TNFRSF1B, low levels of soluble TNFRSF1A were found in sera during our observation period (Fig. 5). A novel mutation (T61I) was detected in the TNFRSF1A gene derived from the genomic DNA of this patient. This is the first case of a TRAPS patient associated with SLE with novel TNFRSF1A mutation.

Many TRAPS families have been reported, and more than 20 different missense mutations, including novel T61I mutations, have been detected between exon 2 and exon 4 in the TNFRSF1A gene [10, 12], although a novel mutation (I199N) which locates in exon 6 was recently reported, and this portion might be involved in receptor shedding [34]. These portions of the receptor encode the cleavage sites in TNFRSF1A for the metalloprotease, TNF-α converting enzyme (TACE) [35–37]. TACE is known to be responsible for generating the soluble homotrimeric form of TNF and the shedding of TNFRSF1A [35, 37]. Although low titres of soluble TNFRSF1A were detected in our patient’s serum, the function of TACE should be intact because TNF production was retained. Many investigators have reported a receptor shedding defect (i.e. H22Y, C30S, C33G, P46L, T50M and C52F mutations) in TRAPS patients [10–12]. Evidence for this includes lack of shedding of soluble TNFRSF1A, as detected by enzyme-linked immunosorbent assays (ELISA), and absence of alteration of TNFRSF1A expression on monocytes and neutrophils after PMA stimulation, as determined by flow cytometry [7, 14, 15]. In our case, although very low titres of soluble TNFRSF1A were detected in her sera during the observation period, the changes in titre of soluble TNFRSF1A correlated with the alterations of titre of TNF and soluble TNFRSF1B (Fig. 5), suggesting that TNFRSF1A was cleaved in response to TNF. This result was supported by experiments showing that normal shedding of TNFRSF1A was
It has been reported that the changes of TNFRSF1A expression on cells in TRAPS patients harbouring the R92Q mutation are the same as the alteration of TNFRSF1A expression in normal individuals [10, 12]. This suggests that defective receptor shedding does not account for the entire pathophysiological mechanism underlying TRAPS. This family study and the high prevalence in the general population show that the T61I mutation has low penetrance, resembling the R92Q mutation [10, 12, 13, 21]. Moreover, a defect in TNFRSF1A shedding in vitro was not observed in monocytes derived from this patient (T61I), as also observed for patients with the R92Q mutation. Recent reports demonstrate that abnormal shedding accounts for a minority of TRAPS patients with the receptor defect, and there is a defect of receptor shedding from monocytes in some families with no TNFRSF1A mutations, indicating that the TRAPS clinical phenotype has a heterogeneous aetiology [13, 21]. After the appearance of TRAPS symptoms, serum levels of soluble TNFRAF1A remained low, whereas serum levels of TNF remained high in this patient (Fig. 5). TNFRSF1A is expressed on the cell surface but large amounts are found localized at the perinuclear–Golgi complex [38]. One possible reason for the low level of soluble TNFRSF1A is that the T61I mutation influences the transport of TNFRSF1A from the Golgi to the cell surface, leading to a low level of TNFRSF1A expression on cells, which correlates with the observed low level of soluble TNFRSF1A in serum after normal cleavage in response to TNF.

Another possibility is that the T61I mutation interferes with TNFRSF1A protein folding and/or trimerization, resulting in low expression of TNFRSF1A. Further experiments comparing patients with the mutation with healthy individuals will be required to differentiate between these possibilities.

It is very important to know whether TNFRSF1A mutations contribute to the development of autoimmune diseases. Our patient was diagnosed with SLE, fulfilling six of the criteria from the revised ACR criteria. We speculate that monocytic fasciitis first appeared in this patient around September 2000; TRAPS symptoms might have arisen in response to TNF production, probably from fascia in both thighs. Hashimoto et al. reported SLE-associated MHC markers in Japanese patients with SLE, observed in monocytes from this patient (Fig. 6). It has been reported that the changes of TNFRSF1A expression on cells in TRAPS patients harbouring the R92Q mutation are the same as the alteration of TNFRSF1A expression in normal individuals [10, 12]. This suggests that defective receptor shedding does not account for the entire pathophysiological mechanism underlying TRAPS. This family study and the high prevalence in the general population show that the T61I mutation has low penetrance, resembling the R92Q mutation [10, 12, 13, 21]. Moreover, a defect in TNFRSF1A shedding in vitro was not observed in monocytes derived from this patient (T61I), as also observed for patients with the R92Q mutation. Recent reports demonstrate that abnormal shedding accounts for a minority of TRAPS patients with the receptor defect, and there is a defect of receptor shedding from monocytes in some families with no TNFRSF1A mutations, indicating that the TRAPS clinical phenotype has a heterogeneous aetiology [13, 21]. After the appearance of TRAPS symptoms, serum levels of soluble TNFRAF1A remained low, whereas serum levels of TNF remained high in this patient (Fig. 5). TNFRSF1A is expressed on the cell surface but large amounts are found localized at the perinuclear–Golgi complex [38]. One possible reason for the low level of soluble TNFRSF1A is that the T61I mutation influences the transport of TNFRSF1A from the Golgi to the cell surface, leading to a low level of TNFRSF1A expression on cells, which correlates with the observed low level of soluble TNFRSF1A in serum after normal cleavage in response to TNF. Another possibility is that the T61I mutation interferes with TNFRSF1A protein folding and/or trimerization, resulting in low expression of TNFRSF1A. Further experiments comparing patients with the mutation with healthy individuals will be required to differentiate between these possibilities.

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with significantly more SLE patients expressing HLA-B39, DRB1*1501, DRB5*0101 and DQB1*0602 than normal controls in Japan [32, 33]. As our TRAPS patient was found to possess three of four susceptibility genes for SLE, she is at higher risk of developing SLE. Aksentijevich et al. speculated that R92Q might have a broader influence on susceptibility to inflammation in early arthritis [10]. We reviewed the charts of five SLE patients with the T61I mutation, and demonstrated that four of the five patients suffered from severe complications which included lupus nephritis (WHO type V) with nephrotic syndrome, lupus nephritis (WHO type V) with haemophagocytic syndrome, autoimmune hepatitis with liver cirrhosis, and interstitial pneumonia with chronic thyroiditis. Interestingly, the T61I mutation exists in 8.3% of SLE patients, whereas T61I was not detected in patients with RA. Nevertheless, no significant differences were detected between SLE and healthy controls in the percentage of the T61I mutation. From these points of view, T61I could influence a generalized pro-inflammatory effect for autoimmune diseases by analogy to R92Q, rather than an additive effect to those provided by HLA-B39, DRB1*1501, DRB5*0101 and DQB1*0602 for susceptibility to SLE. Further investigation of a larger patient and control set for the T61I mutation is clearly needed for determining whether TNFRSF1A mutations contribute to the pathogenesis and development of autoimmune diseases.

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<td>- This is the first report of a TRAPS patient associated with SLE with a novel TNFRSF1A mutation (T61I).</td>
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