Assessment of Type I Interferon Signatures in Undifferentiated Inflammatory Diseases: A Japanese Multicenter Experience

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Research Article

Keywords: Interferon, interferon signature, interferonopathy, autoinflammation, A20 haploinsufficiency, pulmonary hemosiderosis

Posted Date: March 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1285141/v1

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Abstract

Purpose

Upregulation of type I interferon (IFN) signaling has been increasingly detected in inflammatory diseases. Recently, upregulated IFN signature has been suggested as a potential biomarker to identify IFN-driven inflammatory diseases. Yet, it remains unclear to what extent type I IFN is involved in the pathogenesis of undifferentiated inflammatory diseases. This study aimed to quantify the type I IFN signature in clinically undiagnosed patients and assess clinical characteristics in those with a high IFN signature.

Methods

A type I IFN signature was measured in patients’ whole blood cells. Clinical and biological data were collected retrospectively, and an intensive genetic analysis was performed in undiagnosed patients with a high IFN signature.

Results

A total of 113 samples from 94 patients with inflammatory diseases, including 37 undiagnosed cases, were analyzed. Increased IFN signaling was observed in 19 undiagnosed patients, with ten exhibiting clinical features commonly found in type I interferonopathies. Skin manifestations, observed in eight patients, were macroscopically and histologically similar to those found in proteasome-associated autoinflammatory syndrome. Genetic analysis identified novel mutations in the PSMB8 gene of one patient, and rare variants of unknown significance, in genes linked to type I IFN signaling, in four patients. A JAK inhibitor effectively treated the patient with the PSMB8 mutations. Patients with clinically quiescent idiopathic pulmonary hemosiderosis and A20 haploinsufficiency showed enhanced IFN signaling.

Conclusions

Half of the patients examined in this study, with undifferentiated inflammatory diseases, clinically quiescent A20 haploinsufficiency, or idiopathic pulmonary hemosiderosis, had an elevated type I IFN signature.

Introduction

A critical role for type I interferons (IFNs) in the pathogenesis of inflammatory diseases has been increasingly recognized in recent years. IFNs are a group of cytokines that play an important role in host defense against viruses. IFNs consist of three distinct families, namely, type I (IFNα/β/ε/τ/κ/ω/δ/ζ), II (IFNγ), and III (IFNλ). IFNα and IFNβ are the most understood, and broadly expressed type I IFNs. They are produced by most cell types in response to stimulation from pattern recognition receptors through intracellular and endosomal nucleic acids. Once secreted extracellularly, they bind to type I IFN receptors
and activate hundreds of IFN stimulated genes (ISGs), which affect the innate and adaptive immune response[1, 2].

In 2003, multiple investigators reported that peripheral blood cells from systemic lupus erythematosus (SLE) patients demonstrated an overexpression of a characteristic pattern of ISGs, termed an IFN signature[3–5]. Although there is a large overlap between the ISGs induced by all three IFN families, the primary IFN signature is most consistent with induction from type I IFNs[6, 7]. Since detection of type I IFN in human serum by conventional enzyme-linked immunosorbent assay (ELISA) is complicated by low reproducibility and poor correlation with functional assay[8], expression of an IFN signature has been widely used to assess type I IFN activity. An increased IFN signature has been identified in many autoimmune diseases, including SLE, rheumatic arthritis (RA), systemic sclerosis (SSc) and dermatomyositis (DM), and its utility as a biomarker to predict disease severity or to assess disease activity is readily studied [9–16].

Type I IFNs are also involved in the pathogenesis of autoinflammatory diseases. In 2011, Crow et. al. proposed the concept of type I interferonopathy, which refers to a group of Mendelian inflammatory disorders where chronic and autonomous enhancement of type I IFN production was posited as directly relevant to pathogenesis[17]. Since then, numerous Mendelian genotypes were found to be associated with enhanced type I IFN signaling [18]. Several investigators have suggested the utility of a type I IFN signature as a biomarker to distinguish patients with type I interferonopathy from those with other inflammatory diseases[19–22]. Moreover, several groups reported that a type I IFN signature correlates with disease activity and treatment from JAK inhibitors, suggesting that an IFN signature may serve as a biomarker to monitor treatment response[23].

Recent studies have examined the efficacy of JAK inhibitors for the treatment of various autoimmune and autoinflammatory diseases [23–29], suggesting a causal relationship between enhanced type I IFN signaling and disease pathogenesis. Clinically, it is becoming more important to diagnose type I IFN-driven inflammatory diseases rapidly and accurately for personalized medical treatment. In this report, the expression of a type I IFN signature in patients with various inflammatory diseases was investigated. An increased IFN signature was detected in disease states, whose etiologies have not previously been associated with type I IFNs, and in some patients with clinically and genetically undiagnosed inflammatory diseases. In some patients where enhanced IFN signaling was observed, a retrospective assessment of clinical phenotypes revealed characteristics similar to monogenic, type I interferonopathies. In particular, skin manifestations in these cases were macroscopically and histologically similar. These findings may be indicative of a pathogenetic role for type I IFNs in these diseases and suggest the existence of unknown genotypes, which may lead to the upregulation of type I IFN signaling.

Materials And Methods

Patients and healthy controls
Patients, suspected of having autoinflammatory or undifferentiated autoimmune diseases, were selected for this study based on the recommendation of their attending physician. Samples were collected from a total of 94 individuals, comprised of 57 patients with diagnosed inflammatory diseases and 37 patients with undifferentiated inflammatory diseases who had no genetic or clinical diagnosis upon referral. Eleven Japanese adults, who self-reported to have no known medical conditions or infection symptoms, were recruited as healthy controls (HCs). IFN signatures were measured in 124 samples (113 from patients and 11 from HCs) collected between 2016 to 2020.

**Study approval**

The ethics committee of Kyoto University approved this study, which was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from all of the subjects or legally authorized representative.

**Clinical and genetic evaluation**

Clinical and biological data from the 37 patients with undifferentiated inflammatory diseases was collected retrospectively from their medical records or from interviews with the attending physician.

An in-depth genetic analysis was performed on all 19 patients with undifferentiated inflammatory diseases whose type I IFN signature was elevated. Trio-based, whole exome sequencing (WES) was conducted on ten of the patient samples, while targeted genomic sequencing (TS), that analyzed a panel of 533 genes associated with immunodeficiency and autoinflammatory diseases, including monogenic type I interferonopathies, was completed on the other nine patient samples.

**IFN score (IS)**

The IFN signature was measured using quantitative reverse transcription polymerase chain reaction (RT-qPCR) as described previously[19]. Briefly, total RNA was extracted from whole blood using the PAXgene Blood RNA kit (PreAnalytix, Hombrechtikon, Switzerland). Gene expression of six ISGs (IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1) was then determined by RT-qPCR using cDNA derived from 40 ng of total RNA and the TaqMan™ Gene Expression Master Mix (Thermo Fisher Scientific, Waltham, MA), which was run on a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). TaqMan probes for IFI27(Hs01086370_m1), IFI44L(Hs00199115_m1), IFIT1(Hs00356631_g1), ISG15(Hs00192713_m1), RSAD2(Hs01057264_m1) and SIGLEC1(Hs00988063_m1) were used. The relative abundance of each target gene transcript was normalized to the expression level of β actin (HS01060665_g1). The median fold change of the six ISGs, for each patient, was calculated using a ΔCT scale and then compared to the median fold change from the previously collected eleven HCs. IS was calculated as $2^{-\Delta\Delta CT}$. An abnormal IS was defined as being greater than +2 standard deviations above the mean of the control group, i.e., 5.04.

**Statistical analysis**
Descriptive statistical analyses were performed and differences in proportions between the groups in Table 1 were evaluated by a Fisher’s exact test. Results for Fig. E1 were analyzed using a one-way ANOVA with a Dunnett’s multiple comparisons test. A $p$ value of $< 0.05$ was considered significant. All statistical analyses, described above, were performed using the GraphPad Prism software version 8.00 (GraphPad Software, La Jolla, California, USA, www.graphpad.com).
Table 1
Comparison of clinical phenotypes in patients with and without enhanced IFN signaling.

<table>
<thead>
<tr>
<th></th>
<th>Patients with high IS</th>
<th>Patients without high IS</th>
<th>Fisher’s exact test p-value a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>affected / evaluated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=19)</td>
<td>(%)</td>
<td>(n=18)</td>
<td></td>
</tr>
<tr>
<td>Age at onset</td>
<td>8/19 (42.1)</td>
<td>180/180 (100)</td>
<td>0.007</td>
</tr>
<tr>
<td>(median [IQ range], months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7/19 (36.8)</td>
<td>6/13 (35.3)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Fever</td>
<td>14/19 (73.7)</td>
<td>14/18 (77.8)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>14/19 (73.7)</td>
<td>10/18 (55.6)</td>
<td>0.31</td>
</tr>
<tr>
<td>- chilblain</td>
<td>6/19 (31.6)</td>
<td>0/18 (0)</td>
<td>0.02</td>
</tr>
<tr>
<td>- nodular erythema</td>
<td>9/19 (47.4)</td>
<td>3/18 (16.7)</td>
<td>0.08</td>
</tr>
<tr>
<td>Panniculitis</td>
<td>2/19 (10.5)</td>
<td>0/18 (0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Myositis</td>
<td>2/19 (10.5)</td>
<td>0/18 (0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Arthritis / Arthralgia</td>
<td>4/19 (21.0)</td>
<td>3/18 (16.7)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Interstitial pneumoniae</td>
<td>1/19 (5.3)</td>
<td>0/18 (0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>CNS manifestation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- headache</td>
<td>2/19 (10.5)</td>
<td>4/18 (22.2)</td>
<td>0.40</td>
</tr>
<tr>
<td>- intracranial calcification</td>
<td>1/14 (7.1)</td>
<td>0/7 (0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>- aseptic meningitis</td>
<td>0/19 (0)</td>
<td>1/18 (5.6)</td>
<td>0.49</td>
</tr>
<tr>
<td>Transaminitis</td>
<td>7/19 (36.8)</td>
<td>1/18 (5.6)</td>
<td>0.042</td>
</tr>
<tr>
<td>Autoantibody</td>
<td>7/19 (36.8)</td>
<td>2/17 (11.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>- including ANA (1:40 and 1:80)</td>
<td>13/19 (68.4)</td>
<td>3/17 (17.6)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

a; Difference in age at onset was analyzed using a Mann-Whitney test. Two patients from each group whose age of disease onset was ambiguous were excluded from the statistical analysis for this category. All four of these patients self-reported that they had symptoms since early childhood. ANA: antinuclear antibody

Results
The type II IFN signature in clinically or genetically defined cases.

Patients ISs were plotted, according to disease diagnosis, as shown in Figure 1. All patients with monogenic type II interferonopathies, including Aicardi-Goutières syndrome (AGS); proteasome-associated autoinflammatory syndrome (PRAAS); STING-associated vasculopathy with onset in infancy (SAVI); COPA syndrome; spondyloenchondrodysplasia with immune dysregulation (SPENCDI); and other monogenic and polygenic diseases, which are associated with the upregulation of type II IFN signaling, including chronic granulomatous disease (CGD); SLE; and DM; demonstrated high ISs.

A20 haploinsufficiency (HA20) is a systemic autoinflammatory disease caused by a heterozygous loss-of-function mutation in the TNF-α-induced protein 3 (TNFAIP3). Recent reports have demonstrated the elevation of a type I IFN signature and potential therapeutic benefits of JAK inhibitors in HA20 patients [32, 33]. Since nine patients (from six unrelated families) in the study were diagnosed with HA20, their type I interferon signature was analyzed. A determination of the patients’ genotype and TNFAIP3 variants, identified several previously reported variants [30] and detected two variants (p.Lys329Asn*1 and p.Gly583*) that were newly confirmed as pathogenic using the Nuclear factor-kB (NF-κB) reporter gene activity assay (see the method of NF-κB reporter gene activity assay an Figure S1 in the electronic supplemental material (ESM)). All patients had elevated ISs (Figure 1). Intriguingly, when the samples were collected, seven patients were assessed as clinically inactive by the attending physician and the patients reported very few symptoms between hospital visits (Table S1).

Two out of the three patients with idiopathic pulmonary hemosiderosis (IPH) also demonstrated an upregulation of type II IFN signaling (Table S2), despite being in treatment-induced remission for several years. Genetic analyses ruled out COPA syndrome, which is known to be associated with an elevated type II IFN signature and alveolar hemorrhage [32] in two of the patients. Interestingly, one patient with subsequent development of anti-citrullinated protein (ACPA)-positive RA did not have enhanced IFN signaling, while two patients without any autoimmune-related manifestations, other than pulmonary symptoms, demonstrated high ISs.

One patient with chronic active Epstein-Barr virus infection (CAEBV) exhibited moderately enhanced IFN signaling. The type II IFN signature of one patient with hypersensitivity to mosquito bites (HMB) was correlated with the severity of a hydroa vacciniforme-like facial eruption and viral DNA load in whole blood. Interestingly, this patient had a normal type II IFN signature at first, despite the fact that the whole blood viral DNA load was significantly higher than that of the patient with CAEBV (32000 vs. 2400 copy/μg DNA). This demonstrates that the presentation of a normal type II IFN signature cannot exclusively rule out the presence of a chronic viral infection. (Table S3).

Clinical characteristics of patients with undifferentiated inflammatory diseases that exhibited an elevated type II IFN signature.

Of the 37 patients with undifferentiated inflammatory diseases, just over half (19 patients, 51.4%) demonstrated high ISs. A comparison of the clinical features in patients, with and without elevated ISs, is
shown in Table 1. Disease onset was significantly earlier in the patient group with high ISs. The clinical and laboratory features that were more frequently found in the high IS group include, chilblain (31.6% vs. 0%, \( p = 0.0197 \)) and transaminitis (elevation of liver enzymes) (36.8% vs. 5.6%, \( p = 0.0422 \)). While there was no statistical significance, nodular erythema (47.4% vs. 16.7%, \( p = 0.0789 \)) and the presence of autoantibodies (36.8% vs. 11.8%, \( p = 0.1279 \)) were also observed more frequently in patients with high ISs. Of note, low titer antinuclear antibody (ANA) expression (i.e., 1:40 or 1:80), which is usually considered clinically insignificant, was frequently detected in patients with high ISs. Thus, the presence of all autoantibodies, including low titer ANAs, was observed more frequently in patients with high ISs (68.4% vs. 17.6%, \( p = 0.0031 \)).

Amongst the 19 patients with high ISs, ten very early onset cases (< 2 years old, Table 2, P1-10) presented with some symptoms that led to the suspicion they had monogenic type \( \alpha \) interferonopathies, namely, nodular erythema, chilblain-like erythema, panniculitis, myositis, basal ganglia calcification, and interstitial pneumoniae[33][34]. With the exception of P8, all of these patients had similar nodular erythema with post-inflammatory hyperpigmentation, which persisted for weeks to months after resolution, half of which were associated with pain (Figure 2A). Skin biopsy results for all ten patients were available and are shown in Figure 2B. The H&E-stained sections for these patients, with the exception of P7 and P8, showed similar features, consisting of perivascular and periadnexal mononuclear dermal infiltrates with variable positivity for MPO, CD163, and CD3 expression. Most of the MPO-positive infiltrates lacked nuclear segmentation and showed faint CD15 expression, which is usually expressed by mature neutrophils (Figure S2 and Table S4). These findings resembled skin manifestations seen in PRAAS[35]. Patient P3 had two available biopsy results; one specimen (3-1) was taken from a red papule on an upper limb, and the other (3-2) was taken from a painful nodular erythema on a lower limb two years after the first biopsy. Although the immunohistochemistry results were limited, the H&E-stained sections of sample 3-1 shared similar characteristics with the other patients, except for P7 and P8. However, sample 3-2 showed more severe inflammation with dermal infiltration of matured neutrophils, leukocytoclastic vasculitis and septal panniculitis (Figure S2). The microscopic features of the skin specimens from P7 and P8 resembled those seen in SLE (Figure S2). The H&E sample for patient P7 exhibited lobular panniculitis with MPO+/CD15- mononuclear cell infiltration, in addition to superficial and deep perivascular dermatitis with interface vacuolar degeneration. Patient P8's sample showed superficial, dermal CD3-positive T cell infiltration and vacuolar degeneration of the basal layer. MPO+/CD15- mononuclear cells were not identified in P8. These ten early onset patients were strongly suspected of having monogenic type \( \alpha \) interferonopathy. Indeed, one patient was found to have compound heterozygous mutations in \( PSMB8 \), which were proven to be pathogenic, and the other four patients were found to have rare variants, of unknown significance, in genes linked to type \( \alpha \) IFN signaling using a trio-based WES functional assay (manuscript in preparation).

The other nine patients with high ISs, but relatively late disease onset, displayed heterogenous clinical phenotypes (Table 2, P11-19). Patients 11 and 12 had similar clinical features to TAFRO syndrome and idiopathic multicentric Castleman's disease – not otherwise specified (iMCD-NOS), respectively, although their diagnosis was not established since their histopathologic findings were not typical of these
diseases. Patient 11 could have been diagnosed with SLE under the 2019 EULAR/ACR classification criteria; however, the patient’s symptoms were resistant to intensive immunosuppressive treatments targeting SLE, and the patient subsequently died from massive gastrointestinal bleeding at the age of 9[36]. The other seven patients remained clinically and genetically undiagnosed. Three of these patients (P13, 14 and 16) had robust family histories with autoimmune diseases, indicating a highly susceptible genetic background towards autoimmunity or an enhanced IFN response. Also of note, there were three patients (P13, 14 and 17) who suffered from recurrent fever and various accompanying symptoms; however, aside from their high ISs, no other obvious abnormalities were detected. This was despite a thorough work up for fever of unknown origin, and included several imaging studies (CT, MRI, PET-CT), gastrointestinal endoscopy, and bone marrow examination. Interestingly, these three patients showed no significant elevation of inflammatory reactants, such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR), even during febrile episodes; yet, they continued to show constitutively elevated levels of ISs even without apparent symptoms.

**The therapeutic effect of a JAK inhibitor on a pediatric case of PRAAS.**

Patient 1 in Table 2 suffered from a cyclic fever and chilblains-like erythema on the extremities and cheeks, both of which started in early infancy. TS analysis identified a previously reported pathogenic variant (c602G>T, p.Gly201Val) and a novel frame-shift variant (c.389delT, p.129Argfs*27) within the **PSMB8** gene. Compound heterozygosity was confirmed by Sanger sequencing of the patient’s parents. The patient’s clinical manifestations and elevated type  IFN signature were consistent with a diagnosis of PRAAS. Decreased catalytic activity of the immunoproteasome subunit β5i in monocytes from the patient compared to healthy controls confirmed the pathogenicity of the novel frameshift variant (Figure 3A and B, see the method of Proteasome activity detection in the ESM).

Although oral prednisolone (PSL; 1 mg/kg/day) quickly induced remission, the dosage could not be reduced below 0.5 mg/kg/day due to relapse. As the patient became steroid-dependent, baricitinib, a selective JAK1/2 inhibitor, was administered. Oral baricitinib was started at 0.1 mg/kg/day and titrated to 0.38 mg/kg/day. The patient’s spiking fever and myositis resolved and CRP levels decreased to a normal level, corresponding with the dose escalation (Figure 3C and D). The patient’s daily PSL dose was reduced from 0.4mg/kg to 0.15mg/kg. However, discontinuation of the PSL was difficult due to a relapse of occasional low-grade fever and mild erythema, despite the fact that the patient was treated with a significantly higher dose of baricitinib than a typical therapeutic dose for RA (Figure 3C). These results were consistent with a previous report indicating that patients with monogenic interferonopathies require a high dose of baricitinib for treatment [37]. The patient’s ISs correlated well with clinical symptoms (Figure 3C). It is possible that IS-oriented dosing of baricitinib in patients might be helpful for monitoring the therapeutic effect and to avoid overdosing the patient.

**Discussion**
This study identified several patients with clinically and genetically undifferentiated inflammatory disease that had a demonstrated, enhanced IFN signature. Upregulated IFN signaling was also observed in diseases where the association between the etiology and type II IFN has not been completely established, i.e., clinically quiescent HA20 and IPH.

The relatively large number of clinically and genetically undiagnosed patients with enhanced IFN signatures was surprising. Ten undiagnosed patients with upregulated type II IFN signaling presented with very early disease onset (an average of 4.5 months) and possessed some clinical characteristics indicative of monogenic type II interferonopathies. Several shared, unique characteristics were observed in the microscopic features of the patients’ nodular erythema, which resembled histological findings in PRAAS. These findings support the possibility that these patients have some Mendelian genetic defects associated with genes related to type II IFN signaling. Consistent with this theory, a causal mutation was identified in the PSMB8 gene of one patient, and four others were found to have rare variants, of unknown significance, in genes that may be associated with type II IFN signaling. Measuring the IFN signature, in these cases, was useful to narrow down the candidate variants found through genetic analysis. In addition, considering the similarities in type II interferonopathy-like clinical manifestations that may be induced by upregulated type II IFN, it is possible that treatment with JAK inhibitors may be effective in patients without confirmed pathogenic mutations. The possibility of utilizing personalized medicine in patients with undifferentiated inflammatory diseases, based on clinical phenotypes and IFN signatures, to identify patients who will respond to treatment with JAK inhibitors, will be important to determine in future studies.

Several patients with undiagnosed inflammatory diseases that had no symptoms indicative of type II interferonopathies were also identified as having enhanced type II IFN signaling, likely for heterogenous reasons. The diseases in two of these patients were clinically, but not histopathologically, compatible with iMCD-TAFRO and iMCD-NOS respectively. MCD clinical manifestations are believed to be driven by excessive expression of proinflammatory cytokines, particularly IL-6; however, the effectiveness of an IL-6 blockade or other immunosuppressants varies between patients, implying that this syndrome is a heterogenous disease[38, 39]. Several recent reports have indicated that IFN signaling was upregulated in some patients with TAFRO syndrome[40, 41], and an inhibitor of mTOR, a molecule downstream of type II IFN signaling, was effective[42]. Thus, determining whether an IFN signature can be utilized as a biomarker to classify and predict treatment responses in the patients with iMCD is of interest. Some of the patients in the study cohort had a strong family history of autoimmune disease. This is interesting considering the fact that enhanced production of type II IFN has been frequently reported in healthy relatives of SLE patients[43] and in patients with a phase of subclinical autoimmunity[44]. Thus, these patients may be at risk for progression to full blown autoimmune disease in future.

An association between IFN and the etiology of HA20 was first proposed in 2019[45]. Contrary to a previous report[46], an enhanced IFN signature was observed in clinically inactive patients in this study. However, one must consider that these findings are dependent on self-reporting from the patients as well as an assessment of disease activity from the attending physicians, differing from the previous
of type I IFN signaling, as in other monogenic type I interferonopathies. If so, an assessment of the IFN signature in patients will be helpful for diagnosis; for example, when variants of unknown significance are found in the TNFAIP3 gene, as in the present study. A larger study cohort will be necessary to answer this question.

IPH is a rare disorder characterized by diffuse alveolar hemorrhage. Although its etiology remains unknown, the involvement of immunological abnormalities has been suggested based on the presence of autoimmune antibodies[47–49] and the subsequent development of other autoimmune disorders, which have been observed in number of patients with IPH during follow-up[49–52]. Some investigators have suggested that circulating immune complexes deposited into the pulmonary capillaries were involved in the disease pathogenesis[47], which may provoke the upregulation of type I IFN signaling. In this study, two out of three patients diagnosed with IPH had elevated IFN signatures, suggesting a possible link between type I IFN and the etiology of IPH. Further assessment of the type I IFN signature in a cohort of IPH patients may help to characterize this heterogenous disease and provide insight into its etiology. In addition, the IFN signaling pathway could provide a potential target for the treatment of this potentially fatal disorder.

There were some limitations in this study. First, the study cohort was recruited based on the recommendation of the attending physician; therefore, patients with clinical findings indicative of interferonopathies were more likely to be recruited. Second, since the clinical information was collected retrospectively, and not all patients were systematically assessed, limited information was available for some patients. For example, three patients in the study cohort had normal IFN signatures and nodular erythema; however, neither skin images nor histological results were available for these patients. Therefore, no comparison could be made with regard to their skin manifestations and the nodular erythema observed in patients with high IFN signatures. Third, ISs were measured only once in 10 of the 19 patients with high IFN signatures. IFN signatures are known to be elevated during infection; while no evidence of infection was observed during blood sampling, it may be more accurate to repeat the assessment in order to rule out a temporary elevation in IS, especially in patients where a moderate elevation of IFN signature was measured.

Overall, this study demonstrated that a subset of patients exist that have an upregulation of type I IFN signaling without any confirmed disease-causing mutations. Some of these patients may have unknown pathogenic genotypes in genes associated with an upregulation of type I IFN signaling. In some patients, an assessment of the type I IFN signature was useful to narrow down candidate gene variants identified by genetic analysis. The type I IFN signature, in combination with other clinical findings, has the potential to become a useful biomarker for disease diagnosis and treatment choice in the care of patients with inflammatory diseases, although further longitudinal and intervention studies are necessary.
Declarations

Acknowledgments

The authors would like to thank Kumi Kodama for technical assistance; Kazuyoshi Kubo, Takatoshi Tsuchihashi, and Takashi Ishikawa for providing the patients’ clinical information; the Center for Anatomical, Pathological and Forensic Medical Research and the Kyoto University Graduate School of Medicine for preparing the microscope slides; and JAM POST for English language editing assistance.

Funding:

This research was supported by the following grants: 1. A Health Labor Sciences Research Grant for Research on Intractable Diseases from the Ministry of Health, Labor and Welfare (MHLW) of Japan (H29-Nanchi-Ippan-020 and JPMH20317089 to K.I., T.Y., and R.N.); 2. Grants-in-Aids for Young Scientists (grant JP19K17293 to K.I., JP20K16889 to T.S., and JP20K16924 to Y.H.); 3. Grants-in-Aids for Scientific Research (C) (grant JP19K08320 to T.T.); 4. Grants-in-Aids for Scientific Research (B) (grant 19H03620 to S.O.); 5. the Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development (AMED) (JP19ek0109200 and JP20ek0109477 to K.I., 20ek0109387 to K.I. and R.N., JP20ek0109480 to S.O.); 6. a research grant from the Morinaga Hoshikai to K.I., the Core Center for iPS Cell Research of Research Center Network for Realization of Regenerative Medicine from AMED [JP21bm0104001 to M.K.S.]; 7. and the Acceleration Program for Intractable Diseases Research utilizing Disease-specific iPS cells from AMED [17935423 to M.K.S.].

Conflicts of interest/Competing interests:

The authors have no conflicts of interest to declare.

Authors’ contributions:

Takayuki Miyamoto, Yoshitaka Honda., and Kazushi Izawa designed the experiments and authored the manuscript. The first draft of the manuscript was written by Takayuki Miyamoto. Takayuki Miyamoto and Yoshitaka Honda performed the experiments and Takayuki Miyamoto analyzed the data. Takayuki Miyamoto, Yoshitaka Honda, Kazushi Izawa, Nobuo Kanazawa., Hidenori Ohnishi, Takeshi Shiba, Yasuo Nakagishi, Shuji Akizuki, Kosaku Murakami, Masahiro Bamba, Yutaka Nishida, Ayano Inui, Tomoo Fujisawa, Daisuke Nishida, Naomi lwata, Yoshikazu Otsubo, Shingo Ishimori, Motoko Nishikori, Kiminobu Tanizawa, Tomoyuki Nakamura, Takeshi Ueda, Yoko Ohwada, Yu Tsuyusaki, Masaki Shimizu, Takasuke Ebato, Kousho Iwao, Akiharu Kubo, Toshinao Kawai, Tadashi Matsubayashi, Takayuki Tanaka, Satoshi Okada and Takahiro Yasumi collected the clinical data and provided samples (patients and relatives) for the analyses. Masahiko Isa-Nishitani, Hiroshi Nihira, Junya Abe, Eitaro Hiejima, Junko Takita, Ryuta Nishikomori and Takahiro Yasumi provided critical conceptual input and helped author the manuscript. Saori Kadowaki and Hidenori Ohnishi performed the Nuclear factor-kB reporter gene activity assay. Naoya Kase and Megumu K Saito detected proteasome activity. Masakazu Fujimoto, Tatsuhiko Miyazaki and Tomohiro Kanayama prepared microscope slides. Masakazu Fujimoto and Naotomo Kambe performed
microscopic analysis of the skin samples. Osamu Ohara conducted the genetic analysis. All authors reviewed, contributed, and approved the final manuscript.

Data Availability:

The datasets generated and analyzed during the current study are available from the corresponding author upon a reasonable request.

Ethics approval:

This study was conducted in compliance with the Helsinki Declaration. All experiments involving human subjects were conducted in accordance with local regulations and were approved by the Kyoto University Hospital ethics committee (protocol numbers G0432, G0457, G0729, G1091, and G1118).

Consent to participate:

Informed consent was obtained from all participants included in this study.

Consent for publication:

The informed consent document signed by the study participants included granting permission to publish their data.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest

Funding: This research was supported by the following grants: 1. A Health Labor Sciences Research Grant for Research on Intractable Diseases from the Ministry of Health, Labor and Welfare (MHLW) of Japan (H29-Nanchi-Ippan-020 and JPMH20317089 to K.I., T.Y., and R.N.); 2. Grants-in-Aids for Young Scientists (grant JP19K17293 to K.I., JP20K16889 to T.S., and JP20K16924 to Y.H.); 3. Grants-in-Aids for Scientific Research (C) (grant JP19K08320 to T.T.); 4. Grants-in-Aids for Scientific Research (B) (grant 19H03620 to S.O.); 5. the Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development (AMED) (JP19ek0109200 and JP20ek0109477 to K.I., 20ek0109387 to K.I. and R.N., JP20ek0109480 to S.O.); 6. a research grant from the Morinaga Hoshikai to K.I., the Core Center for iPS Cell Research of Research Center Network for Realization of Regenerative Medicine from AMED [JP21bm0104001 to M.K.S.]; 7. and the Acceleration Program for Intractable Diseases Research utilizing Disease-specific iPS cells from AMED [17935423 to M.K.S.].

Conflicts of interest: The authors have no conflicts of interest to declare.

Research involving Human Participants and/or Animals:

Ethics approval: This study was conducted in compliance with the Helsinki Declaration. All experiments involving human subjects were conducted in accordance with local regulations and were approved by the
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**References**


Table 2
Table 2 is available in the Supplemental Files section.

Figures

**Figure 1**

*Patient interferon scores according to disease diagnosis.* Red dots represent an IS greater than 5.04, while blue dots represent ISs below 5.04, based on two standard deviations from the mean score found in healthy controls. Black horizontal lines represent the median for each patient group. DLE: discoid lupus erythematosus; CAEBV: chronic active Epstein-Barr virus infection; HMB: hypersensitivity to mosquito bites; FMF: familial Mediterranean fever; PAAND: pyrin-associated autoinflammation with neutrophilic dermatosis; PFAPA: periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis.
Figure 2

Macroscopic skin manifestations in patients with monogenic interferonopathy-like symptoms. Images of the macroscopic skin manifestations for each patient are shown in panel (a). All patients, with the exception of P8, displayed nodular erythema. These lesions were palpable, sometimes annular, erythematous or violaceous plaques that healed with residual purpura (refer to the lower pictures for P2 and 3). P8 presented with livedo reticularis and skin ulcers. Panel (b) shows H&E-stained sections from the skin lesions. In all patients, with the exception of P7 and P8, mononuclear infiltrates in the perivascular and periannexal dermis were seen. The mononuclear infiltrates in P7 were more intense in deep adipose tissues. Epidermal and superficial dermal infiltrates were observed in P8. The scale bar shown represents 200μm
Figure 3

Imaging findings, proteasome activity and clinical course after baricitinib application in Pt. (a) Ratio of the chymotrypsin-like activity in PBMCs with and without stimulation by TNF-α and IFN-γ. The upregulation of chymotrypsin-like activity in PBMCs from the patient (Pt) was weaker when compared to healthy controls (HCs) upon induction of the immunoproteasome assembly by IFN-γ and TNF-α. (b) Depicts the chymotrypsin-like proteolytic activity of the constitutive proteasome (β5 subunit) and the immunoproteasome (β5i subunit). (c) Transitive graph showing the daily maximum body temperature, C reactive protein levels, and the intermittently measured interferon scores of the patient. (d) A T2-weighted axial MRI depicting high intensity in the femoral muscle before (left) and after (right) treatment with baricitinib

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- electronicsupplementalmaterial.pdf
• Table2.docx