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Subcutaneous injection of interferon gamma therapy could be useful for anti–IFN-γ autoantibody associated disseminated nontuberculous mycobacterial infection

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*ICMJE authorship criteria: All authors meet the ICMJE authorship criteria.

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T.S. was responsible for the organization and coordination of the report. M.H. was the chief investigator and responsible for the data analysis. M.H. and K.F. contributed to the acquisition of the data. All authors contributed to analysis and interpretation of the data. M.H. and K.F. wrote the initial and final drafts of the manuscript. All authors revised the drafts of the manuscript and approved the final version of the manuscript.

Abstract:

One of the human natural defense systems protects against nontuberculous mycobacterial (NTM) infection by IFN-γ producing T lymphocyte cells. Most disseminated NTM infections usually occur in severe immune-compromised patients, such as HIV infection or after organ transplant patients. However, there have been several reports of non-compromised patients with disseminated NTM infection, including antibiotic resistance cases and the presence of a neutralizing antibody against IFN-γ. We elucidated the anti-IFN-γ neutralizing antibody in a 65 year-old Japanese man whose legs were paralyzed because of multiple abscesses in vertebral bodies. Although his vertebral bodies were released due to an operation and antibiotics were administered, this treatment efficacy was poor. Patient's plasma demanded not only IFN-γ expression in peripheral blood mononuclear cells (PBMC) obtained from healthy controls, but also recombinant human IFN-γ□□expression. Furthermore, IFN-γ receptor expression was increased, compared to the healthy control. Finally, anti-IFN-γ antibody was detected in his plasma. These results suggested that anti-IFN-γ antibody induced an incurable NTM infection. IFN-γ□□was subcutaneously administrated with

antibiotics, and then the abscesses diminished and his general condition was successfully improved. This therapy might be useful against severe NTM infections.

Key words:

disseminated nontuberculous mycobacterial infection, anti–IFN-γ autoantibody, interferon gamma therapy

Abbreviations:

NTM: nontuberculous mycobacterial

CAM: clarithromycin

RFP: rifampicin

EB: ethambutol

HIV: human immunodeficiency virus

HTLV-1: human T-cell leukemia virus type 1

AZM: azithromycin

SM: streptomycin

MFLX: moxifloxacin

CPFX: ciprofloxacin

PBMC: peripheral blood mononuclear cells

ICS: intracellular cytokine staining

PMA: phorbol 12-myristate 13-acetate

PHA: phytohemagglutinin

ELISA: enzyme-linked immunosorbent assay

rh-IFN-γ: recombinant human IFN-γ

STAT1: signal transducer and activator of transcription 1

MSMD: mendelian susceptibility to mycobacterial disease

MRI: magnetic resonance imaging

CRP: C-reactive protein

Introduction

IFN-γ cytokine pathway plays an important role in innate and adaptive immune systems against intracellular pathogens [1], which rely on IL-12/23-IFN-γ integrity of macrophages, monocytes and dendritic cells connecting to T lymphocytes or NK cells [2]. The impaired IFN-γ cytokine pathway could permit severe intracellular pathogens infections. In 1995, Casanova et al. reported that mutations in the gene encoding the IFN-γ receptor were the cause of familial susceptibility to mycobacterial infection for the first time [3]. Genetic deficiencies of innate immune signaling lead to intracellular bacterial infections, which is known as Mendelian susceptibility to mycobacterial disease (MSMD) [2, 4]. There have been several recent reports about infection with disseminated NTM in immune-competent patients without a genetic deficiency. The case of neutralizing autoantibodies against IFN-γ cytokine was sometimes intractable, which led to the use of antibiotic therapies [5, 6]. For most patients, the infection occurred when they were around six decades and *Mycobacterium avium* was the dominant pathogen. Autoantibodies against IFN-γ were detected not in pulmonary NTM, but in disseminated NTM patients [7]. Unfortunately, a useful treatment had not been

established; thus, long-term maintenance with antibiotics combination therapy for mycobacterial infection would be necessary [8].

Here, we report an anti–IFN-γ autoantibody-associated disseminated NTM infection case treated with an additional subcutaneous injection treatment of exogenous recombinant IFN-γ and discuss the interferon gamma therapy for disseminated NTM infection with anti–IFN-γ autoantibody.

Case Report

A 65-year-old man with no significant medical history had temporal erythema with nodules on his legs three years before this study, which he had treated with topical steroids. After two years, painful and itchy papules appeared on his legs, and he lost appetite and weight. In addition, wrist and cervical lymph nodes were swollen, so he was administered prednisolone, azathioprine, and an antifungal agent. However, numbness in his lower legs occurred six months after the treatment. A computed tomography scan of vertebral body showed osteolytic changes. Thus, iliac bone biopsy was performed and *Mycobacterium avium* (*M. avium*) was detected in the lesion. He was diagnosed with disseminated nontuberculous mycobacterial infection, stopped azathioprine and started clarithromycin (CAM), rifampicin (RFP), and ethambutol (EB). However, his clinical appearance was not improved and he was hospitalized at our hospital. His body temperature was 37.2 ºC and his bilateral cervical and left side axillary lymph nodes were swollen. His Achilles tendon reflex was increased and his bilateral legs were paralyzed and the skin sensory system under Th4-10 level was disrupted. Laboratory findings on admission are shown in Table 1. There was no

positive fungal antigen, anti-Human Immunodeficiency Virus (HIV) antibody, and anti-Human T-cell Leukemia Virus Type 1 (HTLV-1) antibody. His tuberculin reaction was negative. In interferon-gamma release test, positive control and sample could not be detected. G and M type immunoglobulin were within normal values. Chest X-ray finding was normal (Figure 1A). Computed tomography (CT) findings revealed multiple abscesses on the left-scapula of his backside and around the right-ilium (Figure 1B). Magnetic resonance imaging findings showed osteolytic changes and a fracture on the 10^{th} vertebral bodies (Figure 1C). We performed a transcutaneous biopsy from the abscess around the right ilium, backside of scapula, and a cervical lymph node. All samples revealed to have *M. avium* infection and necrotizing granuloma (Figure 1D, 1E). We diagnosed the disseminated NTM infection. His condition was not improved, despite initial antibacterial treatment with CAM 800 mg/day, RFP 450 mg/day, and EB 750 mg/day. We changed CAM to azithromycin (AZM) 500mg three times a week and added streptomycin (SM) 1 g twice a week and moxifloxacin (MFLX) 400 mg/day. After a month, his symptoms had continued and we changed those antibiotic drugs into CAM 800 mg/day, Rifabutin 300 mg/day, EB 750mg/day, and SM 1 g twice a week

again (Figure 1F). On the other hand, he was in such an immunocompromised state that his tuberculin reaction was negative and the positive control in interferon-gamma release assay could not be detected. Consequently, we hypothesized that the patient had IFN-γ dysfunction and examined it using an *in vitro* test. Firstly, we investigated IFN-γ productivity in peripheral blood mononuclear cells (PBMC) using intracellular cytokine staining (ICS) and flow cytometry after phorbol 12-myristate 13-acetate (PMA) (Merck KGaA, Darmstadt, Germany) and ionomycin (Merck KGaA, Darmstadt, Germany) stimulation. IFN-γ production in PBMC of the patient (PT) was comparable to that in PBMC of the healthy control (HC) (Figure 2A). Second, we investigated IFN-γ receptor 1 (anti-rhIFN-γ receptor 1 antibody, Wako Tokyo, Japan) expression on PBMC using flow cytometry. IFN-γ receptor 1 expression on the PT PBMC was greater than that on HC PBMC (Figure 2B). Next, we separated PBMC and plasma from heparinized peripheral blood in PT and HC. PBMC stimulated using phytohemagglutinin (PHA) (Merck KGaA, Darmstadt, Germany) were co-cultured with the plasma from PT or HC in RPMI 1640 medium including 10% heated-inactivated fetal calf serum. We measured IFN-γ levels in supernatants after 48 hours of incubation using enzyme-linked

immunosorbent assay (ELISA). We divided the IFN-γ level after incubation with PT plasma by the IFN-γ level after incubation with HC plasma, and calculated the relative IFN-γ production ratio in HC and PT PBMC culture. Surprisingly, the incubation with PT plasma remarkably decreased IFN-γ levels in both culture assays (Figure 2C). In addition, to evaluate the neutralizing efficacy against recombinant human IFN-γ (rh-IFN-γ) (Wako, Tokyo, Japan), we examined HC or PT plasma by adding rh-IFN-γ and we measured IFN-γ concentration after an hour of incubation using ELISA. We found that IFN-γ concentration levels in PT plasma were lower than those in HC plasma after the incubation with rh-IFN-γ (PT: 400 pg/mL vs HC: 1,000 pg/mL) (Figure 2D). To evaluate levels of other cytokines related to IFN-γ signaling, we separated PBMC and plasma from PT and HC. PBMC stimulated using lipopolysaccharide (LPS) (Merck KGaA, Darmstadt, Germany) were co-cultured for 40 hours with the plasma from PT or HC. We measured several cytokines levels related to IFN-γ signaling (IL1-β, IL-6, IL-8, IL-10, IL-12 and TNF-α) in the supernatants using ELISA. Those cytokine levels in PT PBMC were equivalent to those in HC PBMC co-cultured with LPS and plasma (Supplementary figure), however level of IL-12 were very low in both supernatants

(data not shown). The results suggested that producing several cytokines related to IFN-γ signaling was intact in the patient and a neutralizing factor against IFN-γ existed in the patient's plasma.

Finally, we confirmed the positive detection of anti-IFN-γ autoantibody 63.5 ELISA unit (E.U.) (cutoff: 0.2 E.U.) in the plasma using ELISA and inhibited mRNA expression of phosphorylated signal transducer and activator of transcription 1 (STAT1) in leukocytes after administration of exogenous recombinant IFN-γ in an *in vitro* assay, according to a previous report [9]. His condition had been unstable and not improved after changing antibiotic drugs. We added a subcutaneous injection of Interferon Gamma-1a 500,000 IU (SHIONOGI & Co., Ltd., Osaka, Japan) three times a week to treatment because we found that low levels of IFN-γ in the patient's plasma had been retained after the incubation with rh-IFN-γ. Thus, we treated the patient with a subcutaneous injection of exogenous recombinant IFN-γ and his symptoms, such as fever, improved and multiple abscesses disappeared after the treatment.

This study was approved by the institutional review board of Hamamatsu University School of Medicine (HUSM 17-318). The patient provided written informed

consent.

Discussion

Recently, disseminated NTM disease in adult healthy patients has been owed to anti–IFN-γ autoantibody. Disseminated NTM without anti–IFN-γ autoantibody develop in immune-compromised patients, such as Human immunodeficiency virus (HIV) patients, while most NTM with anti–IFN-γ autoantibody develop in immune-competent adult patients. Aoki et al. analyzed anti–IFN-γ autoantibody in 331 mycobacterial infection patients and disseminated NTM was identified in 50 cases without HIV infection. Of these, 30 of 37 (81%) immune-competent patients had an increased anti–IFN-γ autoantibody. All cases received prolonged antimicrobial therapy; however, in six cases, antibiotic therapy was resumed after being discontinued [9]. This disease was reported to be difficult to cure, even if an appropriate antibiotic cocktail therapy was administrated. In our case, although endogenous IFN-γ expression was detected in patient's PBMC, IFN-γ levels decreased under the patient's plasma. A similar result was also found in the neutralizing efficacy assay with recombinant human IFN-γ. These results suggested that neutralizing antibody existed in the patient and reflected on the undetectable positive control of QuantiFERON® TB-Gold In-Tube

(QFT-GIT). Regarding IFN-γ mediated immunity, MSMD is also known to be a cause of severe and recurrent bacterial infections [10]. To date, nine MSMD-causing genes have been discovered and most of those gene products are physiologically related to IFN-γ-dependent immunity, including IFN-γ receptor dysfunction [11]. The IFN-γ receptor expression of PBMC in the present case has been higher than that of PBMC in the healthy control. Although IFN-γ receptor coding genes mutations were not analyzed in this case, the function of IFN-γ receptor might be normal because STAT mRNA expression was within the normal range, and the patient had a good response to the subcutaneous injection of IFN-γ therapy.

There is no evidence on why anti–IFN-γ autoantibody is generated after NTM infection; however, most cases occurred in South East Asia [12]. Recently, a study on the interaction between NTM infection and HLA typing has reported that HLA DRB1*16:02 and DQB1*05:02 were found in 82% of patients who have anti–IFN-γ autoantibody [13]. Risk factors associated with detectable anti–IFN-γ autoantibody in NTM infection were HLA class Ⅱ alleles (DRB1 15 or 16, DQB1 05:01, and DQB1 05:02) [7]. It was noted that HLA-DRB1 16:02 and DQB1 05:02 were more prevalent

in the Asian than in the Caucasian population; however, HLA-DRB1 15:01 and DQB1 05:01 were found to be as common among the Caucasian, African American, and South East Asian populations. Although we could not analyze HLA typing information, that might be one of the useful markers for this disease.

In present case, the antibiotics combination therapy against disseminated NTM infection did not work well enough. However, exogenous recombinant IFN-γ treatment had a good response. Our case report patient was East Asian Japanese, but the South East Asian population had a low titer level of anti–IFN-γ autoantibody. Contrary, IFN-γ receptor expression was more increased than in the healthy control. Some reports have also elucidated that highly exogenous IFN-γ levels could overcome an anti–IFN-γ autoantibody neutralizing effect [12, 14]. These findings might be a good response for exogenous recombinant IFN-γ treatment in this case. On the other hand, several other treatments have been reported, such as using anti-CD20 antibody. Browne, et al. treated four patients who have refractory NTM with anti–IFN-γ autoantibody using an anti-CD20 antibody, rituximab, as a treatment [15]. Three out of four patients had already been treated with several antibiotics combinations and exogenous recombinant

IFN-γ before initiation of rituximab treatment. All patients had normal IFN-γ receptor expression and anti–IFN-γ neutralizing level was strong. All patients were treated with rituximab and improved the clinical and laboratory findings, as determined by clearance of infection, resolution of inflammation, reduction of anti–IFN-γ autoantibody levels, and IFN-γ signaling [10]. Anti-CD20 antibody treatment might be one of therapies against NTM with anti–IFN-γ autoantibody.

In summary, NTM with anti–IFN-γ autoantibody is sometimes difficult to cure just by using antibiotics. However, its efficacy for this syndrome might depend on particular HLA-typing, anti–IFN-γ autoantibody neutralizing level, or expression of IFN-γ receptor. These factors are under the complicated mechanisms in the abnormality of IFN-γ immune systems; thus, recognizing anti–IFN-γ neutralizing level or the expression level of other components related to IFN-γ immune systems could be useful for the treatment, such as exogenous recombinant IFN-γ therapy.

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Declarations of Interest

None.

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Ethical approval

Not required as the event described was part of routine service.

Consent

Written consent from the patient was obtained.

Guarantor

Takafumi Suda is a guarantor of this study.

References

[1] van de Vosse E, van Dissel JT and Ottenhoff TH. Genetic deficiencies of innate immune signalling in human infectious disease. The Lancet Infectious diseases. 2009;9:688-98, https://doi.org/10.1016/s1473-3099(09)70255-5

[2] Novelli F and Casanova JL. The role of il-12, il-23 and ifn-gamma in immunity to viruses. Cytokine & growth factor reviews. 2004;15:367-77, https://doi.org/10.1016/j.cytogfr.2004.03.009

[3] Casanova JL, Jouanguy E, Lamhamedi S, Blanche S and Fischer A. Immunological conditions of children with bcg disseminated infection. Lancet (London, England). 1995;346:581,

[4] Ottenhoff TH, Verreck FA, Lichtenauer-Kaligis EG, Hoeve MA, Sanal O and van Dissel JT. Genetics, cytokines and human infectious disease: Lessons from weakly pathogenic mycobacteria and salmonellae. Nature genetics. 2002;32:97-105, https://doi.org/10.1038/ng0902-97

[5] Hoflich C, Sabat R, Rosseau S, Temmesfeld B, Slevogt H, Docke WD, Grutz G, Meisel C, Halle E, Gobel UB, Volk HD and Suttorp N. Naturally occurring anti-ifn-gamma autoantibody and severe infections with mycobacterium cheloneae and burkholderia cocovenenans. Blood. 2004;103:673-5, https://doi.org/10.1182/blood-2003-04-1065

[6] Doffinger R, Helbert MR, Barcenas-Morales G, Yang K, Dupuis S, Ceron-Gutierrez L, Espitia-Pinzon C, Barnes N, Bothamley G, Casanova JL, Longhurst HJ and Kumararatne DS. Autoantibodies to interferon-gamma in a patient with selective susceptibility to mycobacterial infection and organ-specific autoimmunity. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004;38:e10-4, https://doi.org/ 10.1086/380453

[7] Phoompoung P, Ankasekwinai N, Pithukpakorn M, Foongladda S, Umrod P, Suktitipat B, Mahasirimongkol S, Kiertiburanakul S and Suputtamongkol Y. Factors associated with acquired anti ifn- gamma autoantibody in patients with nontuberculous mycobacterial infection. PloS one. 2017;12:e0176342, https://doi.org/10.1371/journal.pone.0176342

[8] Chi CY, Lin CH, Ho MW, Ding JY, Huang WC, Shih HP, Yeh CF, Fung CP, Sun HY, Huang CT, Wu TS, Chang CY, Liu YM, Feng JY, Wu WK, Wang LS, Tsai CH, Ho CM, Lin HS, Chen HJ, Lin PC, Liao WC, Chen WT, Lo CC, Wang SY, Kuo CY,

Lee CH and Ku CL. Clinical manifestations, course, and outcome of patients with neutralizing anti-interferon-gamma autoantibodies and disseminated nontuberculous mycobacterial infections. Medicine. 2016;95:e3927, https://doi.org/10.1097/md.0000000000003927

[9] Aoki A, Sakagami T, Yoshizawa K, Shima K, Toyama M, Tanabe Y, Moro H, Aoki N, Watanabe S, Koya T, Hasegawa T, Morimoto K, Kurashima A, Hoshino Y, Trapnell BC and Kikuchi T. Clinical significance of interferon-gamma neutralizing autoantibodies against disseminated nontuberculous mycobacterial disease. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66:1239-45, https://doi.org/10.1093/cid/cix996

[10] Nekooie-Marnany N, Deswarte C, Ostadi V, Bagherpour B, Taleby E, Ganjalikhani-Hakemi M, Le Voyer T, Rahimi H, Rosain J, Pourmoghadas Z, Sheikhbahaei S, Khoshnevisan R, Petersheim D, Kotlarz D, Klein C, Boisson-Dupuis S, Casanova JL, Bustamante J and Sherkat R. Impaired il-12- and il-23-mediated immunity due to il-12rbeta1 deficiency in iranian patients with mendelian susceptibility to mycobacterial disease. Journal of clinical immunology. 2018;38:787-93, https://doi.org/10.1007/s10875-018-0548-1

[11] Bustamante J, Boisson-Dupuis S, Abel L and Casanova JL. Mendelian susceptibility to mycobacterial disease: Genetic, immunological, and clinical features of inborn errors of ifn-gamma immunity. Seminars in immunology. 2014;26:454-70, https://doi.org/10.1016/j.smim.2014.09.008

[12] Browne SK, Burbelo PD, Chetchotisakd P, Suputtamongkol Y, Kiertiburanakul S, Shaw PA, Kirk JL, Jutivorakool K, Zaman R, Ding L, Hsu AP, Patel SY, Olivier KN, Lulitanond V, Mootsikapun P, Anunnatsiri S, Angkasekwinai N, Sathapatayavongs B, Hsueh PR, Shieh CC, Brown MR, Thongnoppakhun W, Claypool R, Sampaio EP, Thepthai C, Waywa D, Dacombe C, Reizes Y, Zelazny AM, Saleeb P, Rosen LB, Mo A, Iadarola M and Holland SM. Adult-onset immunodeficiency in thailand and taiwan. The New England journal of medicine. 2012;367:725-34, https://doi.org/10.1056/NEJMoa1111160

[13] Chi CY, Chu CC, Liu JP, Lin CH, Ho MW, Lo WJ, Lin PC, Chen HJ, Chou CH, Feng JY, Fung CP, Sher YP, Li CY, Wang JH and Ku CL. Anti-ifn-gamma autoantibodies in adults with disseminated nontuberculous mycobacterial infections are associated with hla-drb1*16:02 and hla-dqb1*05:02 and the

reactivation of latent varicella-zoster virus infection. Blood. 2013;121:1357-66, https://doi.org/10.1182/blood-2012-08-452482

[14] Lee WI, Huang JL, Wu TS, Lee MH, Chen IJ, Yu KH, Liu CY, Yang CH, Hsieh MY, Lin YL, Shih YF, Jaing TH, Huang SC, Kuo TT and Ku CL. Patients with inhibitory and neutralizing auto-antibodies to interferon-gamma resemble the sporadic adult-onset phenotype of mendelian susceptibility to mycobacterial disease (msmd) lacking bacille calmette-guerin (bcg)-induced diseases. Immunobiology. 2013;218:762-71, https://doi.org/10.1016/j.imbio.2012.08.281

[15] Browne SK, Zaman R, Sampaio EP, Jutivorakool K, Rosen LB, Ding L, Pancholi MJ, Yang LM, Priel DL, Uzel G, Freeman AF, Hayes CE, Baxter R, Cohen SH and Holland SM. Anti-cd20 (rituximab) therapy for anti-ifn-gamma autoantibody-associated nontuberculous mycobacterial infection. Blood. 2012;119:3933-9, https://doi.org/10.1182/blood-2011-12-395707

Figure legends

Figure 1. A: Chest X-ray. B: Chest Computed Tomography scan on the breast and pelvis levels. C: Vertebral body magnetic resonance imaging (MRI) on T1 weighted image with enhancement and T2 weighted image. D and E: Histopathological findings, D (upper level): 10th vertebral body species extracted and (bottom level): left cervical lymph node. E: High-power field of each indicated yellow dot region of D. F: Clinical coarse after administration of this case. AZM: azithromycin, CAM: clarithromycin, RFP: rifampicin, Rfb: rifabutin, EB: ethambutol, SM: streptomycin, MFLX: moxifloxacin, CPFX: ciprofloxacin, IFN-γ, solid line represents body temperature (BT) and dash line represents C-reactive protein (CRP).

Figure 2. A: Endogenous IL-4 and IFN-γ production in peripheral blood mononuclear cells (PBMC) extracted from healthy control (HC) and patient (PT) using intracellular cytokine staining and flow cytometry after phorbol 12-myristate 13-acetate and ionomycin stimulation. B: IFN-γ receptor 1 expression on PBMC of HC and PT using a flow cytometer. Anti-CD14 and anti-IFN-γ receptor 1 antibodies were used. C: PBMC

and plasma were separated in HC and PT. PBMC stimulated using phytohemagglutinin were co-cultured with the plasma from HC or PT in RPMI 1640 medium including 10% heated-inactivated fetal calf serum. IFN-γ levels in supernatants after 48 hours of incubation were measured using ELISA. We divided the IFN-γ level after incubation with PT plasma by the IFN-γ level after incubation with HC plasma, and calculated the relative IFN-γ production ratio in HC PBMC culture and PT PBMC culture. D: Neutralizing efficacy against recombinant human IFN-γ in serum. Each serum of the healthy control and the patient was incubated in RPMI1640 medium with recombinant human IFN-γ for an hour at 37 ºC and the serum IFN-γ concentration was measured using ELISA. Black line represents the patient (PT) and gray line represents the healthy control (HC).

Supplementary figure. The cytokines levels related to IFN-γ signaling in the supernatants co-cultured peripheral blood mononuclear cells (PBMC) and plasma from healthy control (HC) and patient (PT) with or without lipopolysaccharide (LPS) stimulation. Those PBMC stimulated using LPS XX ng/ml were incubated with plasma

from HC or PT for 40 hours. The levels of IL1-β, IL-6, IL-8, IL-10, IL-12 and TNF-α in supernatants after incubation were measured using ELISA. Gray bar represents the PBMC from healthy control (HC) and black bar represents the PBMC from patient (PT).

Table 1. Laboratory findings on admission.

Peripheral blood			Serological examination		
WBC	11,300	/μL	CRP	20.8	mg/dL
Neut	80.0	%	RF	8.1	
Lym	10.0	%	IgG	1,556	mg/dL
Eos	3.0	%	IgA	104	mg/dL
Mon	7.0	%	IgM	104	mg/dL
$CD4^+$	626	/μL	IgE	179	IU/mL
$CD8^+$	293	/μL	Na	137	mEq/L
RBC	293	$\times 10^4$/μL	K	4.7	mEq/L
Hb	8.8	g/dL	Cl	96	mEq/L
Ht	27.2	%	Ca	10.1	mg/dL
Plt	28.0	$\times 10^4$/μL	Glu	102	mg/dL
Urinalysis			MPO-ANCA	73	
Blood		(-)	PR3-ANCA	< 10	
Protein		(-)	sIL2-R	8.6	ng/mL
S. pneumonia Ag		(-)			
Legionella Ag		(-)	*Aspergillus Ag*	(-)	
Blood chemistry			*Candida Ag*	(-)	
TP	7.1	g/dL	HCV-Ab	(-)	
Alb	2.9	g/dL	HIV Ab	(-)	
BUN	20.5	g/dL	HTLV-1 Ab	(-)	
Crt	0.75	mg/dL	β-D glucan	< 0.05	pg/mL
T.Bil	0.6	mg/dL			
AST	17	IU/L	BCG reaction	non-reactive	
ALT	15	IU/L	QFT-GIT	undetectable	
LDH	165	IU/L			
ALP	166	IU/L			
PCT	0.74	ng/dL			
CEA	0.5	ng/mL			
ProGRP	24.1	ng/mL			
CYFRA	1.5	ng/mL			
NSE	8.6	ng/mL			

WBC: white blood cells; Neut: neutrophils; Lym: lymphocytes; Eos: eosinophils; Mono: monocytes; RBC: red blood cells; Hb: hemoglobin; Ht: hematocrit; Plt: platelets; TP: total protein; Alb: albumin; BUN: blood urea nitrogen; Crt: creatinine; T.Bil: total bilirubin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; ALP: alkaline phosphatase; PCT: procalcitonin; CEA: carcinoembryonic antigen; ProGRP: progastrin release peptide; CYFRA: cytkeratin 19 Fragment; NSE: ; CRP: C-reactive protein; RF: rhemotoid factore; IgG: immunoglobulin G; IgA: immunoglobulin A; IgM: immunoglobulin M; IgE-RIST:

immunoglonulin E- radioimmunosorbent; Na: sodium; K: potassium; Cl: chlorine; Glu: glucose; MPO-ANCA: myeloperoxidase-anti-neutrophil cytoplasmic antibody; PR3-ANCA: protease3-anti-neutrophil cytoplasmic antibody; sIL-2R: soluble interleukin2-receptor; HCV: hepatitis C virus; HIV: human immunodeficiency virus; HTLV-1: human T-cell leukemia virus type 1; BCG: Bacillus Calmette-Guérin; QFT-GIT: QuantiFERON-TB Gold In-Tube test;

Figure 1

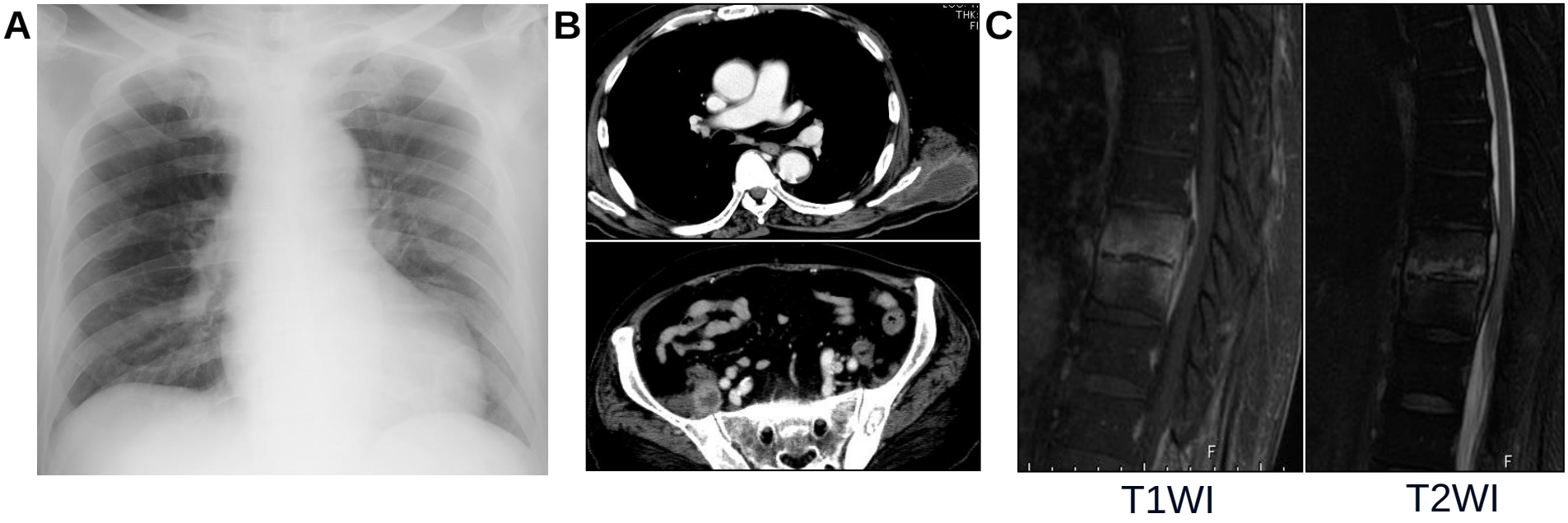
A
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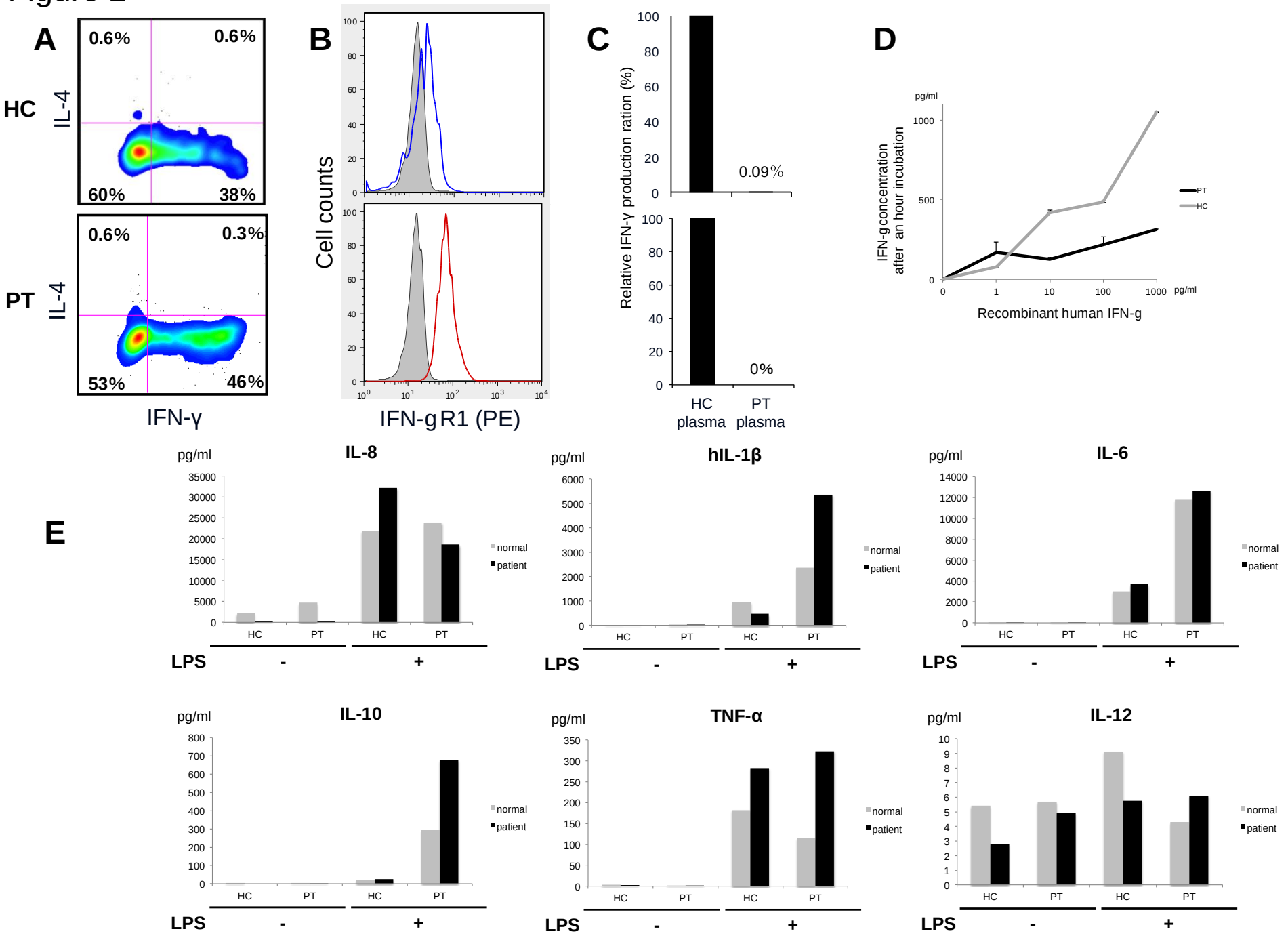
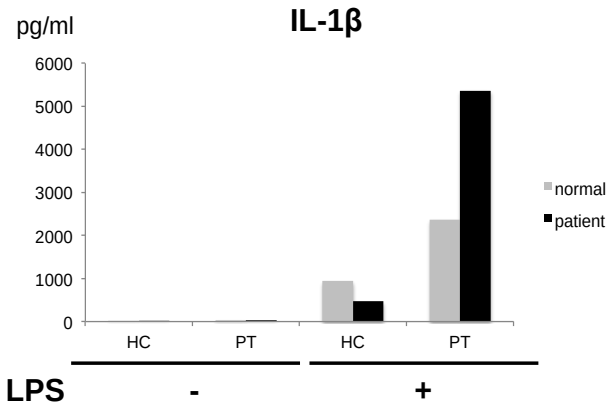
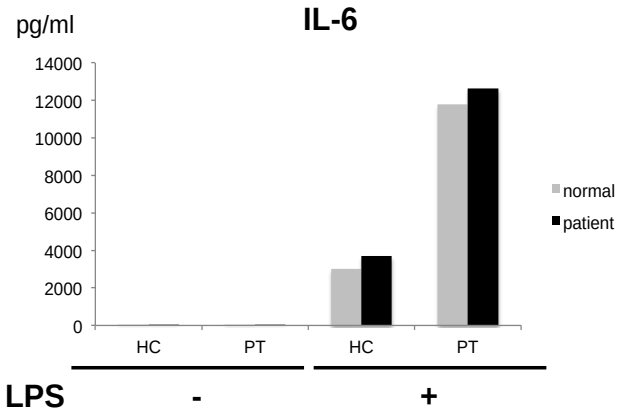
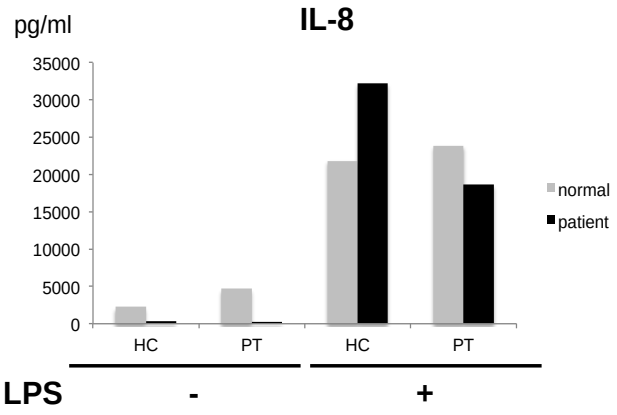
Figure 2

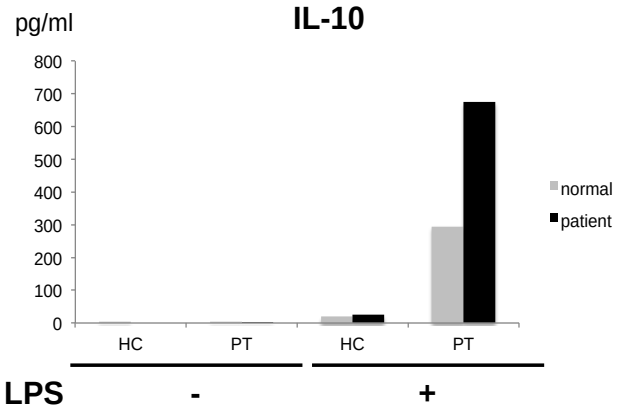

A
HC
PT
IL-4
IFN-γ
0.6%
0.6%
60%
38%
0.6%
0.3%
53%
46%
B
Cell counts
IFN-g R1 (PE)
C
Relative IFN-γ production ration (%)
0.09%
0%
HC plasma
PT plasma
D
pg/ml
IFN-g concentration after an hour incubation
PT
HC
Recombinant human IFN-g
E
IL-8
hIL-1β
IL-6
IL-10
TNF-α
IL-12
pg/ml
normal
patient
HC
PT
LPS
-
+

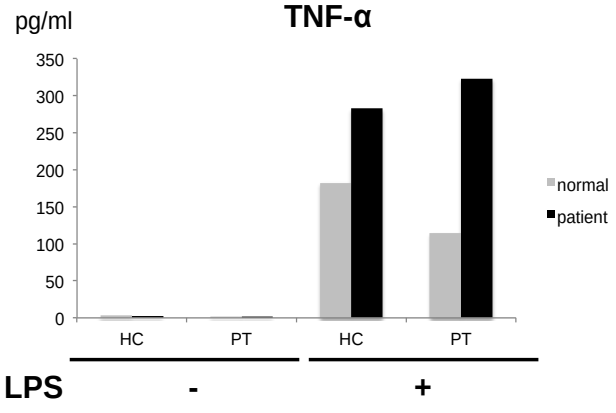
Supplementary figure











Supplementary figure