WILEY
Publishers Since 1807

111 River Street, 8th Floor
Hoboken, NJ 07030, USA
FAX: 201-748-7670

COPYRIGHT TRANSFER AGREEMENT

Date:

To:

Production/Contribution ID#_____ Publisher/Editorial office use only

Re: Manuscript entitled Molecular Analysis of RANKL-Independent Cell Fusion of Osteoclast-Like Cells Induced by TNF-a, Lipopolysaccharide, or Peptidoglycan (the "Contribution")
for publication in Journal of Cellular Biochemistry (the "Journal")
published by Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. ("Wiley").

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the publishing process and enable Wiley to disseminate your work to the fullest extent, we need to have this Copyright Transfer Agreement signed and returned to us as soon as possible. If the Contribution is not accepted for publication this Agreement shall be null and void.

A. COPYRIGHT

1. The Contributor assigns to Wiley, during the full term of copyright and any extensions or renewals of that term, all copyright in and to the Contribution, including but not limited to the right to publish, republish, transmit, sell, distribute and otherwise use the Contribution and the material contained therein in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so.

2. Reproduction, posting, transmission or other distribution or use of the Contribution or any material contained therein, in any medium as permitted hereunder, requires a citation to the Journal and an appropriate credit to Wiley as Publisher, suitable in form and content as follows: (Title of Article, Author, Journal Title and Volume/Issue Copyright © [year] Wiley-Liss, Inc. or copyright owner as specified in the Journal.)

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor's Employer, retains all proprietary rights other than copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution, and the right to make oral presentations of material from the Contribution.

C. OTHER RIGHTS OF CONTRIBUTOR

Wiley grants back to the Contributor the following:

1. The right to share with colleagues print or electronic "preprints" of the unpublished Contribution, in form and content as accepted by Wiley for publication in the Journal. Such preprints may be posted as electronic files on the Contributor's own website for personal or professional use, or on the Contributor's internal university or corporate networks/intranet, or secure external website at the Contributor's institution, but not for commercial sale or for any systematic external distribution by a third party (e.g., a listserve or database connected to a public access server). Prior to publication, the Contributor must include the following notice on the preprint: "This is a preprint of an article accepted for publication in [Journal title] © copyright (year) (copyright owner as specified in the Journal)". After publication of the Contribution by Wiley, the preprint notice should be amended to read as follows: "This is a preprint of an article published in [include the complete citation information for the final version of the Contribution as published in the print edition of the Journal]", and should provide an electronic link to the Journal's WWW site, located at the following Wiley URL: http://www.interscience.Wiley.com/. The Contributor agrees not to update the preprint or replace it with the published version of the Contribution.

2. The right, without charge, to photocopy or to transmit online or to download, print out and distribute to a colleague a copy of the published Contribution in whole or in part, for the Contributor's personal or professional use, for the advancement of scholarly or scientific research or study, or for corporate informational purposes in accordance with Paragraph D.2 below.

3. The right to republish, without charge, in print format, all or part of the material from the published Contribution in a book written or edited by the Contributor.

4. The right to use selected figures and tables, and selected text (up to 250 words, exclusive of the abstract) from the Contribution, for the Contributor's own teaching purposes, or for incorporation within another work by the Contributor that is made part of an edited work published (in print or electronic format) by a third party, or for presentation in electronic format on an internal computer network or external website of the Contributor or the Contributor's employer.

5. The right to include the Contribution in a compilation for classroom use (course packs) to be distributed to students at the Contributor's institution free of charge or to be stored in electronic format in datarooms for access by students at the Contributor's institution as part of their course work (sometimes called "electronic reserve rooms") and for in-house training programs at the Contributor's employer.

D. CONTRIBUTIONS OWNED BY EMPLOYER

1. If the Contribution was written by the Contributor in the course of the Contributor's employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/employer which must sign this Agreement (in addition to the Contributor's signature), in the space provided below. In such case, the company/employer hereby assigns to Wiley, during the full term of copyright, all copyright in and to the Contribution for the full term of copyright throughout the world as specified in paragraph A above.

2. In addition to the rights specified as retained in paragraph B above and the rights granted back to the Contributor pursuant to paragraph C above, Wiley hereby grants back, without charge, to such company/employer, its subsidiaries and divisions, the right to make copies of and distribute the published Contribution internally in print format or electronically on the Company's internal network. Upon payment of the Publisher's reprint fee, the institution may distribute (but not resell) print copies of the published Contribution externally. Although copies so made shall not be available for individual re-sale, they may be included by the company/employer as part of an information package included with software or other products offered for sale or license. Posting of the published Contribution by the institution on a public access website may only be done with Wiley's written permission, and payment of any applicable fee(s).

E. GOVERNMENT CONTRACTS

In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government Employees: see note at end).

F. COPYRIGHT NOTICE

The Contributor and the company/employer agree that any and all copies of the Contribution or any part thereof distributed or posted by them in print or electronic format as permitted herein will include the notice of copyright as stipulated in the Journal and a full citation to the Journal as published by Wiley.

G. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work. If the Contribution was prepared jointly, the Contributor agrees to inform the co-Contributors of the terms of this Agreement and to obtain their signature to this Agreement or their written permission to sign on their behalf. The Contribution is submitted only to this Journal and has not been published before, except for "preprints" as permitted above. (If excerpts from copyrighted works owned by third parties are included, the Contributor will obtain written permission from the copyright owners for all uses as set forth in Wiley's permissions form or in the Journal's Instructions for Contributors, and show credit to the sources in the Contribution.) The Contributor also warrants that the Contribution contains no libelous or unlawful statements, does not infringe on the rights or privacy of others, or contain material or instructions that might cause harm or injury.

CHECK ONE:

[_X_]Contributor-owned work

Contributor's signature _____ Date _____

Hitoshi Hotokezaka, PhD, DDS

Type or print name and title

Co-contributor's signature _____ Date _____

Type or print name and title

ATTACH ADDITIONAL SIGNATURE PAGE AS NECESSARY

[____]Company/Institution-owned work (made-for-hire in the course of employment)

Company or Institution (Employer-for-Hire) _____ Date _____

Authorized signature of Employer _____ Date _____

[____]**U.S. Government work**

Note to U.S. Government Employees

A Contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication is called a "U.S. Government work," and is in the public domain in the United States. In such case, the employee may cross out Paragraph A.1 but must sign and return this Agreement. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[____]**U.K. Government work (Crown Copyright)**

Note to U.K. Government Employees

The rights in a Contribution prepared by an employee of a U.K. government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown. In such case, the Publisher will forward the relevant form to the Employee for signature.

Eur Respir J 2007; 29: 1217–1223
DOI: 10.1183/09031936.00061806

Serological test and chest computed tomography findings in patients with *Mycobacterium avium* complex lung disease

S. Kitada*, Y. Nishiuchi#, T. Hiraga*, N. Naka*, H. Hashimoto*, K. Yoshimura*, K. Miki*, M. Miki*, M. Motone*, T. Fujikawa*, K. Kobayashi", I. Yano+ and R. Maekura*

ABSTRACT: The present authors have previously reported the usefulness of a serodiagnostic test to detect serum glycopeptidolipid (GPL) core antibody in diagnosing *Mycobacterium avium* complex (MAC) lung disease in immunocompetent patients. The aim of the present study was to investigate correlations between the levels of antibody against GPL core and chest computed tomography (CCT) findings in patients with MAC lung disease.

A total of 47 patients with MAC-positive culture from their sputum and who had radiographic abnormalities were investigated. Thirty-three patients met the American Thoracic Society criteria for MAC disease; 14 did not. All patients underwent both CCT examination and the serodiagnostic test for MAC at the same time.

Small nodular shadows were seen on CCT in all 47 patients and bronchiectasis shadows were seen in 39 (83%) of them. There was a significant positive correlation between the extent of the disease and the level of GPL core immunoglobulin (Ig)A antibody. The levels of GPL core IgA antibody were significantly elevated in patients who had nodular shadows (10–30 mm) compared with patients who had small nodular shadows (<10 mm).

The present results document that the levels of immunoglobulin A antibody against glycopeptidolipid core correlate with the chest computed tomography findings of *Mycobacterium avium* complex lung disease.

KEYWORDS: Early stage, enzyme immunoassay, glycopeptidolipid, mycobacteria

It has long been recognised that *Mycobacterium avium* complex (MAC) is an important pathogen causing chronic pulmonary infection in immunocompetent individuals [1] and that the incidence of the disease has increased recently in Japan [2] and other countries [1, 3]. The diagnosis and management of MAC lung disease is therefore becoming a matter of increasing concern among respiratory physicians.

The present authors previously reported the usefulness of a serological test for diagnosis of MAC lung disease with a glycopeptidolipid (GPL) core antigen that was used for enzyme immunoassay [4]. The GPL core is a common structure of the GPL antigen, which is a major cell surface antigen in MAC and which is not present in the cell wall of either *M. tuberculosis* complex or *M. kansasii* [5, 6]. The present authors examined the usefulness of the GPL serodiagnostic test in immunocompetent patients with lung disease and found that MAC lung disease could be clearly differentiated from colonisation with MAC and from lung diseases caused by either *M. tuberculosis* or *M. kansasii*. The sensitivity and specificity of the test for diagnosing MAC lung disease were 92.5 and 95.1%, respectively, for immunoglobulin (Ig)A. Combining this serodiagnostic test with the criteria advocated by the American Thoracic Society (ATS) for nontuberculous mycobacterial respiratory disease in 1997 [7] facilitated easier and more rapid definitive diagnosis of MAC lung disease.

Moreover, the levels of GPL core antibodies reflected disease activity because they decreased in MAC patients responding to chemotherapy [4]. However, correlations between levels of GPL core antibody and radiographic findings have not been evaluated thus far. Therefore, the present authors assess herein the levels of GPL core antibody in relation to chest computed tomography (CCT)

AFFILIATIONS
*Dept of Internal Medicine, National Hospital Organization Toneyama National Hospital, and
#Toneyama Institute for Tuberculosis Research, Osaka,
"Dept of Immunology, National Institute of Infectious Diseases, and
+Japan BCG Laboratory, Tokyo, Japan.

CORRESPONDENCE
S. Kitada
Dept of Internal Medicine
National Hospital Organization
National Toneyama Hospital
5-1-1 Toneyama
Toyonaka-shi
Osaka 560-8552
Japan
Fax: 81 668533127
E-mail: kitadas@toneyama.hosp.go.jp

Received:
May 09 2006
Accepted after revision:
January 09 2007

STATEMENT OF INTEREST
None declared.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

findings in patients with MAC-culture positive sputum whose radiographic findings were infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules.

MATERIAL AND METHODS

Study subjects

A total of 47 patients were enrolled at the National Hospital Organization (NHO) National Toneyama Hospital (Osaka, Japan) between September 2001 and May 2004. They fulfilled the following criteria: 1) MAC-positive cultures from sputum; 2) abnormal shadows that were infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules on their chest radiographs; and 3) no predisposing lung disease. Patients were divided into two groups (the MAC disease group and the MAC-culture positive group) based on the guidelines advocated by the ATS (table 1) [7]. The individuals who had a single and small amount of culture-positive MAC but did not have clinical symptoms and had no abnormal lesions on CCT findings were excluded from the present study as contaminated respiratory specimens. These cases did not have evidence of active disease.

Of the 47 patients with MAC-positive cultures, 30 met the ATS criteria at enrolment. Patients who did not meet these criteria were followed up for 12 months with monthly radiographic and sputum examination with Ziehl–Neelsen stains and cultures on Ogawa egg medium. Three patients met the criteria; 14 patients still had not over the 12-month follow-up period after enrolment. Based on these observations, the subjects were divided into the MAC disease group, which was composed of 33 patients who met the ATS criteria, and the MAC-culture positive group, which was composed of 14 patients who did not. All patients underwent CCT examination and a serodiagnostic test at the same time. These took place when the diagnosis of MAC lung disease was made in the MAC disease group or when the follow-up period ended in the MAC-culture positive group. Clinical data were collected from each patient at the time of computed tomography (CT); these included sex, age, body mass index, smoking history, drinking history, complications, past history and laboratory data, including erythrocyte sedimentation rate, and GPL core IgG, IgA and IgM antibody. The present authors investigated whether there was a correlation between GPL core antibody level and CCT findings. Of the MAC disease group, 15 patients had previously received combination chemotherapy for mycobacterial diseases recommended by the ATS guideline before enrolment, but they had positive cultures of MAC at enrolment. All patients were seronegative for HIV types 1 and 2. Informed consent was obtained from all patients. The present study was approved by the NHO National Toneyama Hospital institutional review board for experimentation on human subjects and complies with international guidelines for studies involving humans.

CCT findings

All patients underwent conventional CT examination. CCT scans were obtained using a Toshiba Asteion TSZ-021A CT scanner (Toshiba, Tokyo, Japan). The CCT findings were categorised into small nodular shadow (<10 mm), nodular shadow (10–30 mm), large nodular shadow (>30 mm) or infiltrate, bronchiectasis, cavity and atelectasis. CCT findings were assessed by a consensus reading performed by two individual respiratory physicians without prior knowledge of the clinical or laboratory data. To assess the extent of disease,

TABLE 1 Criteria for diagnosis of *Mycobacterium avium* complex lung disease

Clinical criteria	1) Compatible signs and symptoms (coughing, fatigue more common; weight loss, haemoptysis and shortness of breath may be present, particularly in advanced disease) with documented deterioration of the patient's clinical state if a base condition is present, and
	2) Reasonable exclusion of other disease (e.g. tuberculosis, cancer, histoplasmosis) to explain condition or adequate treatment of other condition with increasing signs/symptoms
Radiographic criteria	1) Any of the following chest radiographic abnormalities; if baseline films are >1 yr old, should be evidence of progression:
	Infiltrates with or without nodules (persistent for >2 months or progressive)
	Cavitation
	Nodules alone (multiple)
	2) Any of the following high-resolution computed tomography abnormalities:
	Multiple small nodules
	Multifocal bronchiectasis with or without small lung nodules
Bacteriological criteria	1) At least three available sputum/bronchial wash samples within 1 yr, as follows:
	Three positive cultures with negative AFB smears, or
	Two positive cultures and one positive AFB smear; or
	2) A single available bronchial wash and inability to obtain sputum samples, as follows:
	Positive culture with 2+, 3+ or 4+ growth, or
	Positive culture with a 2+, 3+ or 4+ AFB smear; or
	3) Tissue biopsy, as follows:
	Any growth bronchopulmonary tissue biopsy
	Granuloma and/or AFB on lung biopsy with one or more positive cultures from sputum/bronchial wash
	Any growth from usually sterile extrapulmonary site

For a diagnosis of pulmonary disease, all three criteria (clinical, radiographic and bacteriological) must be satisfied. AFB: acid-fast bacilli.

the lung was divided into 18 segments on conventional CCT according to the anatomical segment as follows. Right upper lobe (RUL) apical segment, RUL posterior segment, RUL anterior segment, middle lobe (ML) lateral segment, ML medial segment, right lower lobe (RLL) superior segment, RLL medial basal segment, RLL anterior basal segment, RLL lateral basal segment, RLL posterior basal segment, left upper lobe (LUL) apicoposterior segment, LUL anterior segment, lingular superior segment, lingular inferior segment, left lower lobe (LLL) superior segment, LLL anterior medial basal segment, LLL lateral basal segment and LLL posterior basal segment. The extent of the lesions was expressed as the number of involved CCT segments in which MAC lesions were present.

GPL core antibody

GPL core antibody was measured as previously described [4]. Briefly, microtitre plates (Nunc Products, Roskilde, Denmark) were coated with 0.5 $\mu g \cdot well^{-1}$ of GPL core of *M. avium* serotype 4, which had been prepared according to a previously described method [4]. Serum samples were diluted 40-fold with PBS containing 1% bovine serum albumin. Diluted serum samples were added, followed by incubation for 1 h at 37°C. Plates were washed, then peroxidase-conjugated F(ab')2 of goat antibody against human IgG, IgA or IgM (Sigma, St. Louis, MO, USA) was added and plates were incubated for 2 h at 37°C. Unbound labelled antibody was removed by washing and the substrate, *o*-phenylenediamine dihydrochloride (Sigma), was added. Following colour development, the optical densities (OD) of the wells on the plates were read for absorbance at 492 nm (model 550; Bio-Rad Laboratories, Tokyo, Japan).

Statistical analysis

All data were analysed and all values are given as mean ± SD. The Mann–Whitney U-test was used to compare the differences between groups. The Chi-squared test was used to compare the difference in CCT findings between groups. Correlation coefficients were calculated using Spearman's rank method. A p-value of <0.05 was considered significant.

RESULTS

Clinical background and laboratory data

The clinical background and laboratory data are shown in table 2. A total of 33 patients met the ATS criteria (MAC disease group) and 14 patients did not (MAC-culture positive group). Of these patients, 46 were female, who tended to be thin, there was only one smoker and none were alcohol abusers or had severe systemic complications. The main symptoms were coughing (19 patients), sputum (21 patients), bloody sputum (nine patients), chest pain (four patients) and dyspnoea (three patients). Of the subjects, 40.4% had past histories of major surgery that included myomectomy (seven patients), appendectomy (five patients), mastectomy (three patients), cholecystectomy (two patients), gastrectomy (two patients) and oophorectomy (one patient). There were no statistically significant differences in clinical characteristics between the two groups.

Levels of GPL core antibody

IgG, IgA and IgM antibodies specific for GPL core antigen were measured (fig. 1). The levels of IgG against GPL core antigen were 0.219 ± 0.292 OD for MAC disease and 0.268 ± 0.372 OD for the MAC-culture positive group. The values for IgA were 0.547 ± 0.438 OD and 0.452 ± 0.345 OD, respectively, and for IgM 0.628 ± 0.362 OD and 0.535 ± 0.213 OD, respectively. Applying the cut-off value 0.064 OD for IgG, 0.072 OD for IgA and 0.312 OD for IgM in the present authors' previous study [4], the positive rate was 66.7% for IgG, 81.8% for IgA and 78.1% for IgM in the MAC disease group, and 71.4% for IgG, 100% for IgA and 84.6% for IgM in the MAC-culture positive group. There were no statistically significant differences between the MAC disease and MAC-culture positive groups for any Ig isotype.

CCT findings

The CCT findings are summarised in table 3. Abnormal CCT were similar in the MAC disease and MAC-culture positive groups, with the exception of findings related to large nodules or infiltrate, which were more frequent in the former ($p<0.05$). Small nodules <10 mm in diameter were seen in all patients. Analysis of the distribution of the lesions showed that MAC frequently involved the ML lateral segment (33 out of 47 patients, 70.2%), ML medial segment (33 patients, 70.2%) and lingular inferior segment (30 patients, 63.8%). The mean numbers of involved segments in each finding were similar regardless of large nodules or infiltrate. The total numbers of involved segments were 6.7 ± 4.2 and 5.0 ± 4.3 in the MAC disease group and the MAC-culture positive group, respectively. From these results of clinical characteristics, serodiagnosis using GPL core antibody and CCT findings, it could be considered that the patients of the MAC-culture positive group had an active MAC lung disease.

Correlation between CCT findings and level of GPL core antibody

Table 4 shows the correlation coefficients between the numbers of involved CCT segments, representing the extent of disease, and the level of GPL core antibody in the MAC disease group and the MAC-culture positive group. There is a significant positive correlation between the extent of disease and the level of GPL core IgA antibody in both groups (fig. 2). Next, the level of GPL core antibody was compared with each CCT finding, including the occurrence of a small nodular shadow (<10 mm), a nodular shadow (10–30 mm), a large nodular shadow (>30 mm) or infiltrate, bronchiectasis and atelectasis. The levels of GPL core IgA antibody were significantly elevated in patients who had nodular lesion(s) (≥10 mm) compared with patients who had small nodular lesion(s) (<10 mm) in both groups (fig. 3). There were no differences in GPL core antibody levels correlating with other findings. These results document that a higher level of GPL core IgA indicated a wider extent of MAC disease and larger nodule formation on CCT.

DISCUSSION

The present study is the first to assess a correlation between GPL core antibody levels and radiographic findings. A total of 47 patients with MAC-positive culture from sputum and abnormal shadow on chest radiographs were examined. The present authors found that the level of IgA antibody against GPL core antigen was associated with CCT findings: a higher level of GPL core IgA antibody indicated a wider extent of MAC disease and larger nodule formation on CCT. Obviously, in order to establish this new knowledge, further studies with a

TABLE 2. Clinical data of patients with *Mycobacterium avium* complex (MAC)-positive cultures

	MAC disease	MAC-culture positive
Subjects n	33	14
Sex male/female	1/32	0/14
Age yrs	65.3±10.6	71.4±6.0
BMI kg·m^{-2}	19.2±3.0	18.5±2.2
Cigarette smoking	0	1
Alcohol abuse	0	0
Past history of major surgery	15 (45.5)	4 (28.5)
ESR mm·h^{-1}	33.2±24.7	26.7±17.3
MAC species		
M. avium	21	12
M. intracellulare	9	2
Both	3	0

Data are presented as n or mean±SD. BMI: body mass index; ESR: erythrocyte sedimentation rate.

larger number of patients are required due to the low value of the correlation coefficients between the extent of disease and levels of GPL core IgA (r=0.514) and a small number of study subjects.

The ATS criteria published in 1997, consisting of clinical, radiographic and bacteriological criteria, are the best guide to diagnosis and treatment of pulmonary disease caused by nontuberculous mycobacteria, including MAC [7]. All three elements are required for the diagnosis of MAC disease. The bacteriological criterion requires multiple positive cultures for MAC, or a positive culture from a lung biopsy or histologically proven lung biopsy positivity. In the present study, it was not possible to carry out lung biopsy or bronchial washings on all patients in the MAC-culture positive group, especially on those with minimal symptoms or on elderly subjects, because informed consent for the bronchoscopic examination had not been obtained. Bronchoscopic examination is invasive and expensive. In elderly patients, the diagnosis for MAC lung disease may be less important with respect to the long-term survival because, for MAC lung disease patients with progressive radiographic abnormalities, the 50% survival rate was 175 months [8]. Thus, MAC-culture positive patients were defined based on an observation with monthly radiographic and sputum examination for 12 months.

Most patients in the MAC-culture positive group were elderly, nonsmoking, thin females with no severe systemic complications; these clinical features are consistent with those of patients with MAC lung disease with nodular bronchiectasis [9]. The combination of multiple small nodules on CCT with bronchiectasis, particularly in the middle lobe and/or lingual lobe, should suggest the diagnosis of MAC lung disease [10–12]. In the present study, the clinical background and laboratory findings, including GPL core antibody, were similar between the MAC disease group and the MAC-culture positive group. A GPL core IgA antibody was positive in all patients of the MAC-culture positive group. Moreover, all patients in the

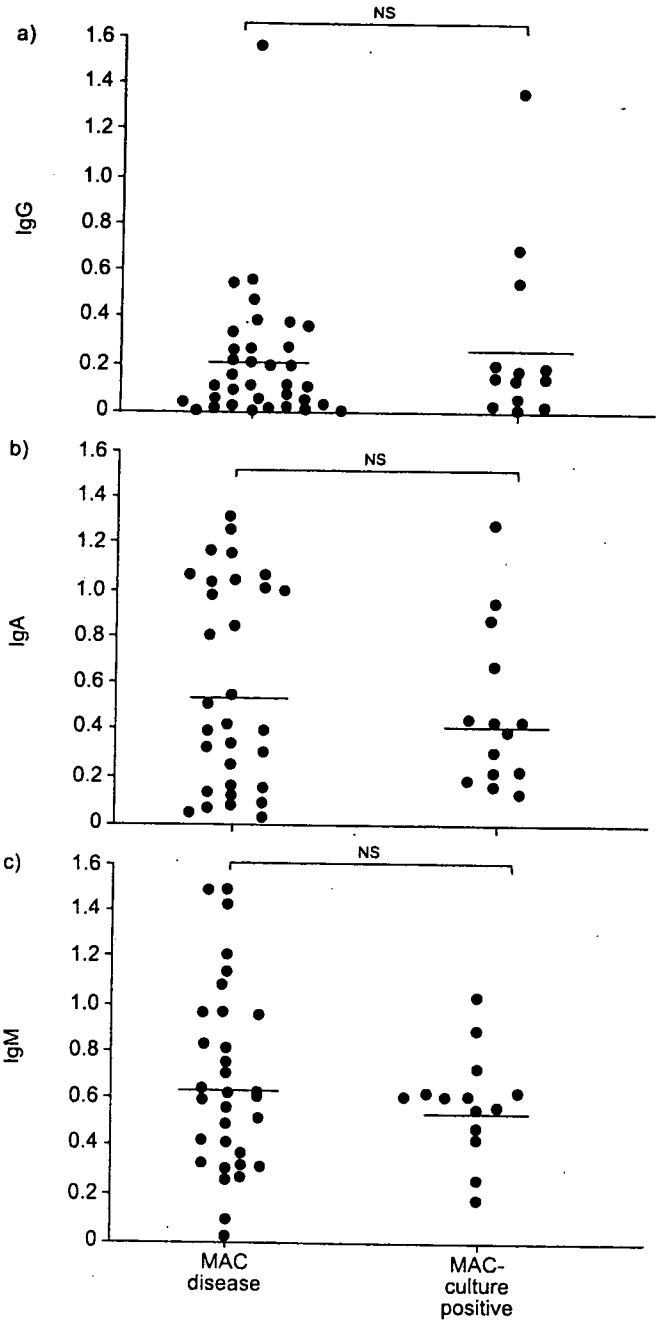

FIGURE 1. The distribution of serum levels of glycopeptidolipid core antibody in patients with *Mycobacterium avium* complex (MAC) disease and in the MAC-culture positive group in the a) immunoglobulin (Ig)G, b) IgA and c) IgM groups. The mean of each group of values is indicated by a horizontal line. There was no statistically significant difference between the IgG, IgA and IgM groups. NS: nonsignificant.

MAC-culture positive group had findings of small nodules on CCT. Some careful investigation of CCT and histological findings revealed that small nodular lesions were caused by granulomas formed as a specific response to mycobacterial infection [13, 14]. Furthermore, the individuals with MAC colonisation, who had a single and small amount of culture-positive MAC but did not have clinical symptoms or abnormal lesions on CCT findings, were excluded from the present study

TABLE 3 Chest computed tomography findings

Abnormalities	MAC disease		MAC-culture positive	
	Patients with findings	Involved segments	Patients with findings	Involved segments
Small nodule (<10 mm)	33 (100)	6.5±4.2 (1–17)	14 (100)	4.3±3.7 (1–12)
Nodule (10–30 mm)	17 (51.5)	1.2±1.3 (1–4)	3 (21.4)	0.7±1.5 (0–4)
Large nodule (>30 mm) or infiltrate*	8 (24.2)	0.5±1.3 (0–5)	0 (0)	0
Bronchiectasis	29 (87.9)	3.1±2.5 (0–14)	10 (71.4)	2.7±3.1(0–10)
Cavity	4 (12.1)	0.3±1.1 (0–6)	2 (14.3)	0.2±0.6 (0–2)
Atelectasis	13 (39.4)	0.5±0.7 (0–2)	3 (21.4)	0.2±0.4 (0–1)
Other	4 (12.1)	0.2±0.6 (0–3)	0 (0)	0
Total	33 (100)	6.7±4.2 (1–17)	14 (100)	5.0±4.3 (1–12)

Data are presented as n (%) or mean±SD (range). MAC: *Mycobacterium avium* complex. *: $p<0.05$.

at enrolment. The present authors have previously reported that GPL core antibody was not detectable in cases of MAC colonisation [4]. From results, it was considered that the patients of the MAC-culture positive group had an active MAC lung disease. It could be argued that patients are suffering from MAC lung disease when they: 1) exhibit a positive respiratory culture for MAC; 2) have chest radiographic findings of infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules; and 3) show positive results for GPL core antibody. When using GPL core antibody it is not necessary to continue collecting respiratory specimens for acid-fast bacilli analysis or to observe chest radiographs and/or CCT over a 12-month period of time.

In the MAC disease group, eight patients had a large nodular shadow (>30 mm) or infiltrate, whereas none in the MAC-culture positive group had these findings. Thus, the MAC-culture positive group might be considered to represent early stage MAC disease because serial CCT scanning in MAC lung disease has shown that the development of infiltrate is preceded by the appearance of nodules [15]. It might be recommended for patients in both groups to be administered with immediate multidrug chemotherapy and/or surgical therapy in the context of the patient's general condition and

TABLE 4 Correlation coefficients between numbers of involved chest computed tomography segments *versus* glycopeptidolipid (GPL) core antibody level

	Correlation coefficients		
	Total	MAC disease	MAC-culture positive
GPL core IgG antibody OD	0.150	0.070	0.268
GPL core IgA antibody OD	0.514***	0.487**	0.788*
GPL core IgM antibody OD	0.217	0.306	0.153

MAC: *Mycobacterium avium* complex; Ig: immunoglobulin; OD: optical density. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

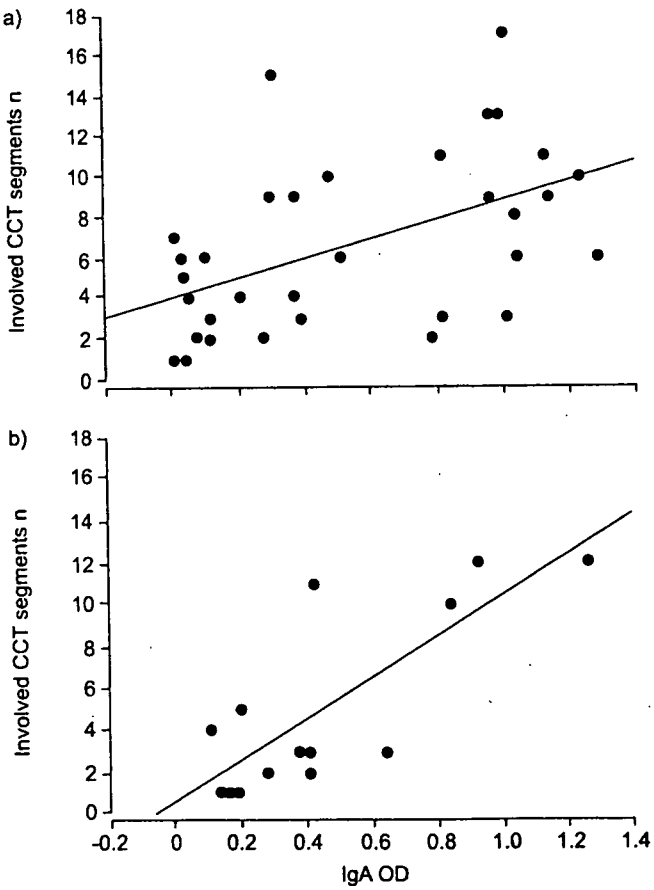

FIGURE 2. Correlation between the levels of glycopeptidolipid (GPL) core-specific immunoglobulin (Ig)A antibody and the number of involved chest computed tomography (CCT) segments in patients with a) *Mycobacterium avium* complex (MAC) lung disease and b) in the MAC-culture positive group. Significant positive correlations were found between the level of GPL core IgA antibody and the number of involved computed tomography segments in the MAC disease group ($r=0.487$, $p<0.01$), in the MAC-culture positive group ($r=0.788$, $p<0.05$), and in both of them ($r=0.514$, $p<0.001$). OD: optical density.

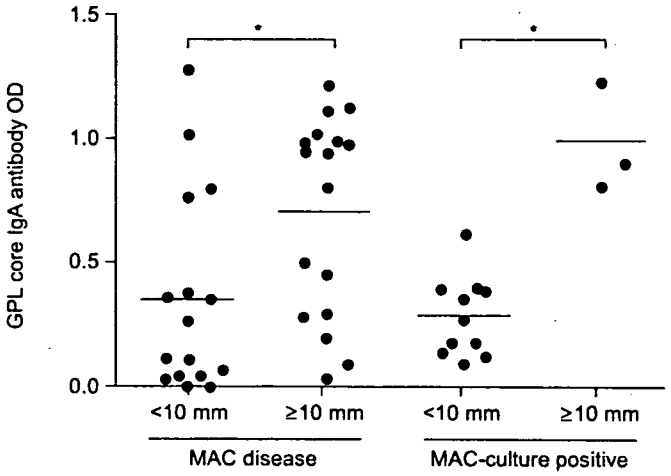

FIGURE 3. Serum level of glycopeptidolipid (GPL) core immunoglobulin (Ig)A antibody in patients who had nodular lesions <10 mm or ≥10 mm in diameter assessed by chest computed tomography in the *Mycobacterium avium* complex (MAC) disease group and in the MAC-culture positive group. The mean of each group is indicated by a horizontal bar. The levels of GPL core IgA antibody were significantly elevated in patients who had nodular lesions (≥10 mm) compared with patients who had small nodular lesions (<10 mm) in both the MAC disease group ($p<0.05$) and in the MAC-culture positive group ($p<0.05$), and the total of them ($p<0.001$). OD: optical density. *: $p<0.05$.

tolerance to the medication. However, 12 out of 33 patients with MAC disease and nine out of 14 MAC-culture positive patients did not undergo multidrug chemotherapy, including clarithromycin, following the present study. This was because most of these patients were >70 yrs old and/or did not have substantial symptoms and/or advanced or progressive radiographic abnormalities. Furthermore, treatment for MAC lung disease is expensive and is not covered by healthcare insurance in Japan. MAC lung disease is also difficult to treat and recurrence frequently occurs in MAC disease patients, even after completing multidrug chemotherapy, including clarithromycin. Many cases of recurrence have been experienced by the present authors, with the smear or culture test being positive over the 12 months following sputum-negative conversion during chemotherapy. This is because the radiographic active lesions, which are bronchiectasis or a cavity, have usually remained at the time of the sputum-negative conversion [8]. Thus, rapid diagnosis and treatment are required at an early stage before the completion of bronchiectasis or cavity lesions.

The serodiagnostic test used in the present study to detect serum GPL core antibodies could add useful information as a supplementary diagnostic aid [4, 16] and the present authors believe that this test may have future diagnostic applications. However, to include this serodiagnostic test in routine clinical practice, a study addressing the correlation between the antibody levels and radiographic findings was needed; the present study fulfils this requirement. The positive rates of the serological test were 71.4% for IgG, 100% for IgA and 84.6% for IgM in the MAC-culture positive group. If this serological test is combined with the ATS criteria, a better sensitivity to diagnose MAC lung disease without lung biopsy might be obtained.

The levels of GPL core antibody were similar in the MAC disease group and the MAC-culture positive group. Fifteen out of 33 (45.5%) of the MAC disease patients had received combination chemotherapy recommended by the ATS guidelines [7]. It is possible that this might have affected their antibody levels. However, the effects of treatment might be limited because they had a positive culture of MAC at enrolment, which meant the chemotherapy was not successful in converting the culture result from positive to negative at the time of serum sample collection. In the present authors' previous study, unsuccessful chemotherapy did not affect the level of GPL core antibody [4].

The level of IgA, but not IgG or IgM, GPL core antibody was significantly associated with the radiographic findings of the disease, but the reasons for this remain unclear. IgA is the predominant immunoglobulin isotype in mucosal tissue and is believed to be involved in the defence against viral and bacterial infection at this site. There are some published reports that are consistent with the present findings. RODRIGUEZ *et al.* [17] reported that IgA may play an important role in protection against mycobacterial infection in the respiratory tract by blocking the pathogens' entrance and/or by modulation of pro-inflammatory responses. In the present authors' previous study [4], the best serodiagnostic results for sensitivity and specificity for diagnosing MAC lung disease were obtained by measuring IgA. Moreover, WATANABE *et al.* [18] reported that total serum IgA was significantly higher in patients with MAC compared with those with pulmonary tuberculosis. These reports indicate that IgA antibody might play an important role in the chronic inflammation of mucous membrane of the respiratory tract in patients with MAC lung disease. The role of GPL core IgA antibody in protection against MAC is not clear and further studies are needed to address this question.

In summary, the present article documents that the level of immunoglobulin A glycopeptidolipid core antibody was significantly associated with radiographic findings. This observation should encourage the use of the serodiagnostic tests for *Mycobacterium avium* complex lung disease in clinical practice.

ACKNOWLEDGEMENTS

The authors wish to thank K. Maekura, Y. Yamamoto and M. Kobayashi for measurement of antibody levels.

REFERENCES

1 Field SK, Fisher D, Cowie RL. *Mycobacterium avium* complex pulmonary disease in patients without HIV infection. *Chest* 2004; 126: 566–581.
2 Sakatani M. The non-tuberculous mycobacteriosis. *Kekkaku* 2005; 80: 25–30.
3 Marras TK, Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med* 2002; 23: 553–567.
4 Kitada S, Maekura R, Toyoshima N, *et al.* Use of glycopeptidolipid core antigen for serodiagnosis of *Mycobacterium avium* complex pulmonary disease in immunocompetent patients. *Clin Diagn Lab Immunol* 2005; 12: 44–51.

5 Brennan PJ, Nikaido H. The envelope of mycobacteria. *Annu Rev Biochem* 1995; 64: 29–63.
6 Aspinall GO, Chatterjee D, Brennan PJ. The variable surface glycolipids of mycobacteria: structures, synthesis of epitopes, and biological properties. *Adv Carbohydr Chem Biochem* 1995; 51: 169–242.
7 American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. Medical Section of the American Lung Association. *Am J Respir Crit Care Med* 1997; 156: S1–S25.
8 Maekura R, Okuda Y, Hirotani A, *et al.* Clinical and prognostic importance of serotyping *Mycobacterium avium–Mycobacterium intracellulare* complex isolates in human immunodeficiency virus-negative patients. *J Clin Microbiol* 2005; 43: 3150–3158.
9 Prince DS, Peterson DD, Steiner RM, *et al.* Infection with *Mycobacterium avium* complex in patients without predisposing conditions. *N Engl J Med* 1989; 321: 863–868.
10 Swensen SJ, Hartman TE, Williams DE. Computed tomographic diagnosis of *Mycobacterium avium-intracellulare* complex in patients with bronchiectasis. *Chest* 1994; 105: 49–52.
11 Primack SL, Logan PM, Hartman TE, Lee KS, Muller NL. Pulmonary tuberculosis and *Mycobacterium avium-intracellulare*: a comparison of CT findings. *Radiology* 1995; 194: 413–417.
12 Wittram C, Weisbrod GL. *Mycobacterium avium* complex lung disease in immunocompetent patients: radiography–CT correlation. *Br J Radiol* 2002; 75: 340–344.
13 Jeong YJ, Lee KS, Koh WJ, Han J, Kim TS, Kwon OJ. Nontuberculous mycobacterial pulmonary infection in immunocompetent patients: comparison of thin-section CT and histopathologic findings. *Radiology* 2004; 231: 880–886.
14 Fujita J, Ohtsuki Y, Suemitsu I, *et al.* Pathological and radiological changes in resected lung specimens in *Mycobacterium avium intracellulare* complex disease. *Eur Respir J* 1999; 13: 535–540.
15 Obayashi Y, Fujita J, Suemitsu I, Kamei T, Nii M, Takahara J. Successive follow-up of chest computed tomography in patients with *Mycobacterium avium-intracellulare* complex. *Respir Med* 1999; 93: 11–15.
16 Kitada S, Maekura R, Toyoshima N, *et al.* Serodiagnosis of pulmonary disease due to *Mycobacterium avium* complex with an enzyme immunoassay that uses a mixture of glycopeptidolipid antigens. *Clin Infect Dis* 2002; 35: 1328–1335.
17 Rodriguez A, Tjarnlund A, Ivanji J, *et al.* Role of IgA in the defense against respiratory infections IgA deficient mice exhibited increased susceptibility to intranasal infection with *Mycobacterium bovis* BCG. *Vaccine* 2005; 23: 2565–2572.
18 Watanabe K, Fujimura M, Kasahara K, *et al.* Characteristics of pulmonary *Mycobacterium avium-intracellulare* complex (MAC) infection in comparison with those of tuberculosis. *Respir Med* 2003; 97: 654–659.

HIV感染症における結核感染診断に対してのQuantiFERON®-TB第2世代の有用性についての検討

永井　英明　川辺　芳子　有賀　晴之　鴫山　文子
島田　昌裕　久能木真喜子　松井　芳憲　川島　正裕
鈴木　純子　大島　信治　益田　公彦　松井　弘稔
田村　厚久　長山　直弘　赤川志のぶ　町田　和子
倉島　篤行　四元　秀毅

要旨：〔目的〕リンパ球のIFN-γ産生能を測定することによって結核感染の診断を行う方法（QFT-2G）が開発された。細胞性免疫機能が著しく低下するHIV感染症では，判定不可例の増加，感度の低下等が予想されるので，HIV感染症例におけるQFT-2Gの有用性について検討した。〔対象と方法〕①HIV感染症合併結核例，②抗HIV療法を受けているHIV感染症例，の2群につき，QFT-2G，CD4数，ツ反応等を検討した。〔結果〕①HIV感染症合併結核例13例では，QFT-2Gの感度は76.9%でツ反応の感度：発赤38.5%（硬結15.4%）に比べ有意に高かった。判定不可例が1例ありCD4数は16/μlと最も低い症例であった。②抗HIV療法施行中のHIV感染者25例にQFT-2Gを行い，判定不可例はなかった。CD4数は100～1157/μlであり，非結核既往群ではQFT-2G陽性はなく，結核既往群では陽性は3例（27.3%）であった。〔結論〕HIV感染症においてQFT-2GはCD4数の著減例では判定不可となる可能性があった。HIV感染症合併結核におけるQFT-2Gの感度は高く，十分有用であると考えられた。結核既往者の中にQFT-2G陽性者がおり，結核の再燃が起こるのか注意深い観察が必要である。

キーワーズ：結核，HIV感染症，QuantiFERON-TB第2世代，ESAT-6，CFP-10

はじめに

従来，結核感染の診断はツベルクリン反応（ツ反応）によって行われてきた。この方法はBCG未接種者においては感度，特異度ともに高く基本的には優れた方法であるが，BCG接種者においては，現れる反応が過去のBCG接種によるものか，最近受けた結核感染によるものかが区別できないという大きな問題がある。BCG接種に積極的に取り組んできたわが国では，結核感染の有無をツ反応で判定するのはしばしば困難を極める。そこにBCG接種の影響を受けない新しい結核診断法が開発された。特異的抗原刺激に対するリンパ球のインターフェロンγ（IFN-γ）産生能を測定することによって結核感染の診断を行う方法（QuantiFERON®-TB第2世代，以下QFT-2G）である。

QFT-2Gは，結核菌由来の特異抗原early secreted antigenic target 6（ESAT-6）とculture filtrate protein 10（CFP-10）の刺激による末梢血リンパ球のIFN-γ産生能を測定する検査法で，結核感染の診断有用性は高い。Moriらりによれば[1]QFT-2Gの結核感染の診断における特異度は98.1%，感度は89%である。

しかしながら，免疫抑制状態ではQFT-2Gの感度は低下する可能性があり，その有用性についての検討は乏しい。特に細胞性免疫機能が著しく低下するHIV感染症では，QFT-2Gの判定不可例の増加，結核を合併した場合のQFT-2Gの感度の低下が予想される。そこで，①結核発病時のHIV感染症例，②外来通院中のHIV感染症例（結核の既往例を含む）についてQFT-2Gを行い，

独立行政法人国立病院機構東京病院呼吸器科

連絡先：永井英明，独立行政法人国立病院機構東京病院呼吸器科，〒204-8585 東京都清瀬市竹丘3-1-1
(E-mail: hnagai-in@tokyo-hosp.jp)
(Received 22 Feb. 2007/Accepted 9 Apr. 2007)

QFT-2Gの判定不可例の頻度，結核発病時の感度等について検討した。

方　法

①対象は結核菌を確認できたHIV感染症合併結核のうち，結核の治療開始直前か，治療開始後1週間以内にQFT-2Gを行えた症例である。結核の既往歴がある症例およびすでに抗HIV薬の投与を受けている症例は除いた。対象症例は13例あり，男性12例，女性1例であった。年齢は20～67歳（中央値53歳）であった。

②対象は当院外来通院中で強力な抗HIV療法（highly active antiretroviral therapy: HAART）を行っていたHIV感染症例である。結核既往群と非結核既往群に分けて検討した。結核既往群は，結核診断時にHIV陽性と判明した症例で，当院で結核の治療を開始し，その後HAARTを開始した症例である。結核は治癒し，結核の治療を終了し外来にてHAART施行中に対象とした。非結核既往群は病歴上，結核の既往および結核患者との接触が明らかでない症例である。

外来通院中のHIV感染者のうちQFT-2Gを測定できた症例は25例あり，結核既往群11例（男女比9：2，29～58歳：中央値49歳），非結核既往群14例（男女比14：0，年齢31～65歳：中央値55歳）であった。

①，②の症例につきQFT-2G，CD4陽性Tリンパ球（CD4）数，ツ反応等について検討し，QFT-2Gの判定不可例の有無，QFT-2Gとツ反応との感度の比較等を行った。

ツ反応は発赤では発赤径10 mm以上を陽性とし，硬結ではATS/CDCの基準[2]によりHIV感染者の場合，硬結径5 mm以上を陽性とした。QFT-2Gの判定基準は次項に記載した。

QFT-2Gの感度とツ反応の感度の比較には，Fisher's exact probability testを用いた。

なお，QFT-2Gは2006年4月に保険収載となったが，それ以前の検査については当院倫理委員会の承認を得て，説明と同意のうえ行われた。

QFT-2G

Moriらの方法に準じた。すなわち，被験者から静脈血をヘパリン加採血し，12時間以内にその一定量にESAT-6抗原，CFP-10抗原，陰性コントロールとしての生理的食塩水，陽性コントロールとしてのマイトジェン（phytohemagglutinin: PHA）を添加し，16～24時間37℃で培養した。培養後に上清を採取し，サンドイッチ酵素免疫測定法（ELISA法）でIFN-γの濃度を測定した。

刺激抗原ESAT-6，CFP-10により産生誘導されたIFN-γ値から陰性コントロールのIFN-γ産生値を差し引いた値のうち高値を選択した。0.35 IU/ml以上を陽性，0.1 IU/ml未満を陰性とした。その間の0.1以上0.35 IU/ml未満は判定保留とした。また，結核特異抗原によるIFN-γ産生値が0.35 IU/ml未満で，陽性コントロールから陰性コントロールを差し引いた値が0.5 IU/ml未満の場合は細胞性免疫応答が低下しているものとし，特異的免疫応答による測定値には信頼性がないとして，判定不可とした。

結　果

①QFT-2Gを行えたHIV感染症合併結核例13例の結核病変は，粟粒結核6例，肺結核6例，リンパ節結核1

Table 1 Results of QFT-2G in tuberculosis patients with HIV infection

Case	Gender/Age	CD4 counts (/μl)	Tuberculin skin test (mm) induration/erythema (double erythema)	QFT-2G
1. Miliary TB	M/45	16	0×0/3×3	Indeterminate
2. Miliary TB	M/60	23	0×0/0×0	Positive
3. Pulmonary TB	M/59	27	0×0/0×0	Intermediate
4. Pulmonary TB	M/57	36	0×0/0×0	Positive
5. Pulmonary TB	M/47	48	11×10/61×41	Positive
6. Miliary TB	M/53	60	0×0/0×0	Intermediate
7. Miliary TB	F/38	63	0×0/15×13	Positive
8. Pulmonary TB	M/66	68	0×0/0×0	Positive
9. Miliary TB	M/63	81	0×0/5×5	Positive
10. Pulmonary TB	M/36	101	0×0/0×0	Positive
11. Miliary TB	M/67	199	0×0/15×15	Positive
12. Lymph node TB	M/41	245	15×17/20×20 (40×57)	Positive
13. Pulmonary TB	M/20	320	0×0/16×21	Positive
	Median 53	Median 63	Sensitivity erythema 38.5% (induration 15.4%)	Sensitivity 76.9%

TB: tuberculosis

Table 2 Results of QFT-2G in HIV-infected patients under HAART

	Number	QFT-2G			CD4 counts (/μl)
		Positive	Intermediate	Negative	Median (range)
Past history of TB (Completion of TB therapy)	11	3	2	6	348 (124–561)
No history of TB	14	0	2	12	496 (100–1157)

TB: tuberculosis

Table 3 Results of QFT-2G in HIV infected persons after completing of TB therapy

	QFT-2G	Gender/Age	Duration between QFT-2G and TB diagnosis (months)	CD4 counts (/μl) (at TB diagnosis)	Tuberculin skin test (induration: mm) (at TB diagnosis)
1.	Positive	F/40	19	218 (63)	20 (0)
2.	Positive	M/42	48	234 (11)	13 (0)
3.	Positive	F/29	67	396 (72)	25 (6)
4.	Intermediate	M/57	12	124 (27)	12 (0)
5.	Intermediate	M/58	93	367 (423)	8 (0)
6.	Negative	M/46	30	178 (2)	9 (0)
7.	Negative	M/55	42	320 (106)	16 (0)
8.	Negative	M/43	50	549 (188)	13 (31)
9.	Negative	M/53	55	348 (31)	8 (0)
10.	Negative	M/50	77	561 (111)	13 (0)
11.	Negative	M/49	85	518 (35)	0 (0)
	Positivity rate 27.3%	Median 49	Median 50	Median 348 (63)	Positivity rate 90.9 (18.2) %

TB: tuberculosis

例であった（Table 1）。CD4数は16～320/μl（中央値63/μl）であった。QFT-2Gの結果は，陽性：13例中10例（76.9%），判定保留：13例中2例（15.4%），判定不可：13例中1例（7.7%）であった。ツ反応の陽性率は発赤で判定した場合38.5%，硬結で判定した場合15.4%であった。QFT-2Gの感度はツ反応硬結の感度よりも有意に高かった（$p<0.01$）。

判定不可例のCD4数は16/μlと最も低値であった。この症例はHAARTを開始後，CD4数が増加し，陽性コントロールが認められるようになった。しかし，その時点のQFT-2Gは陰性であった。

② 外来通院中のHIV感染者のうちQFT-2Gを測定できた症例25例は，全例にHAARTが施行され，CD4数は結核既往群124～561/μl（中央値348/μl），非結核既往群100～1157/μl（中央値496/μl）であり，CD4数が著しく低下している例はなかった（Table 2）。いずれも陽性コントロールに対するIFN-γ産生は良好で，判定不可例はなかった。非結核既往群14例ではQFT-2G陽性はなく，判定保留2例，陰性12例であった。結核既往群11例（Table 3）では，HAARTによりCD4数が増加（中央値63→348/μl）し，ツ反応は硬結陽性率が18.2%から90.9%へ上昇した。しかし，QFT-2G陽性者は3例（27.3%）であり，ツ反応の陽性率に比べ低かった。判定保留2例，陰性6例であった。

考　察

われわれの対象症例数は少なかったが，HIV感染者においてもCD4数が著しく低下していなければQFT-2Gは判定不可とならず判定可能であると判明した。また，HIV感染者における活動性結核の発病時においては，QFT-2Gはツ反応に比べ陽性率がより高く，結核感染診断の有用性がきわめて高いと考えられた。

結核感染の診断はツ反応で行われてきたが，ツ反応の反応性は細胞性免疫機能に左右される。活動性結核であっても細胞性免疫機能が低下した状態ではツ反応の陽性率は低下し，偽陰性例が増加する。特に細胞性免疫機能が著しく低下するHIV感染症では，ツ反応の診断能力は低下する。Johnsonら[3]によれば活動性結核を合併したHIV感染者の30%，AIDS発病例の60%以上はツ反応硬結径が10 mm以下であったという。したがって，HIV感染症では結核感染の診断法としてツ反応の有用性は低下する。そこで，近年開発され結核感染の診断において高い感度を示すIFN-γ産生能を測定する方法が期待される。

IFN-γ測定法を用いてHIV感染症における結核診断を行う際に注意が必要な点は，細胞性免疫機能が低下しているので，PHAによる陽性コントロールが確実に得られるかという点である。これが得られない場合は判定

不可となる。当院の症例では結核合併例における判定不可例が1例（7.7%）あり，この症例は13例中CD4数が最も低値（16/μl）であった。やはり免疫機能が著しく低下した症例は判定不可となる可能性があるので，この点については認識しておくべきである。しかし，外来通院中のHAART施行例25例におけるQFT-2G検査では，判定不可例はなかった。25例のCD4数値は100～1157/μl（中央値396/μl）であり，CD4数が著しく低下していなければQFT-2Gは判定不可にならないと考えられた。

Brockら[4]によれば590名のHIV感染者にQuanti-FERON-TB In-Tube test（In-Tube version）を行ったところ，陽性者には潜在性結核感染症のリスクをもった症例や結核の既往のある症例が多かった。しかし，CD4数が少ないほど判定不可例が多かった。

QFT-2Gと同様にESAT-6およびCFP-10の両特異抗原を利用してリンパ球を刺激し，反応性のIFN-γの産生を測定するT-SPOT.TBという方法がある。これはIFN-γ産生測定法としてenzyme-linked immunospot（ELISPOT）assayを用いる方法である。T-SPOT.TBについてはHIV感染症においても十分にPHAに反応し，CD4数に影響を受けないという報告[5]がある。QFT-2GとT-SPOT.TBを比較した報告[6]では，判定不可例はQFT-2G：11%，T-SPOT.TB：3%とQFT-2Gのほうが多かった。特に5歳以下ではQFT-2Gでは判定不可が多かった。南アフリカのHIV感染症も結核も非常に多い地域での活動性結核を発病していない160名（HIV陽性者74名，陰性者86名）に対して，T-SPOT.TB，QFT-2G，ツ反応を施行した報告[7]がある。HIV陽性者は陰性者に比べ，ツ反応の陽性率は有意に低かった。しかし，T-SPOT.TB（陽性率：HIV陽性者52%，HIV陰性者59%）もQFT-2G（陽性率：HIV陽性者43%，HIV陰性者46%）も陽性率にHIV陽性・陰性に差がなかった。両者とも中等度に進んだHIV感染症では感度が落ちないとしている。ただし，判定不可例はELISPOTで1%，QFT-2Gで7%認められている。上記の報告[6][7]をみるとT-SPOT.TBに比べ，QFT-2Gは免疫能が低下した状態では判定不可例が生じやすい可能性があるので注意が必要である。

当院のAIDS合併結核におけるQFT-2Gの結核感染診断の感度は76.9%であり，ツ反応に比べ有意に高率であり，HIV感染症においても結核感染の診断には有用な検査法と考えられた。また，判定保留症例が2例あったが，いずれもCD4数が27/μl，60/μlと低値であることを考慮すると，この2例においても結核感染を示している可能性が高い。免疫低下状態における判定保留症例の扱いについてはさらに症例を集め検討すべきである。

HIV感染症合併結核におけるIFN-γ産生能測定法の有用性についての報告は少なく，ELISPOTについての報告が散見されるのみである。Chapmanら[8]は39例のHIV感染症合併結核おけるELISPOTの感度は90%と非常に高く，有用であったと報告している。Liebeschuetzら[9]によるAfricaの小児の前向き研究では，ELISPOTの結核診断の感度は83%であり，ツ反応の感度の63%に比較し有意に高かった。免疫機能が低下していると考えられる3歳以下の小児，HIV感染症，低栄養状態における結核感染に対するツ反応の感度は51%，36%，44%であった。これに対してELISPOTはそれぞれ，85%，73%，78%と高値であり，ELISPOTは免疫機能の低下にも影響を受けにくいという結果であった。この2報告はELISPOTについてであるが，当院のHIV感染症合併結核におけるQFT-2Gの感度76.9%はほぼ同等の結果であった。今後さらに症例を増やし検討したい。

ELISPOTはHIV感染者においても判定不可例が少なく期待される検査法であるが，QFT-2Gに比べ検査法が煩雑であり，現時点では容易にわが国で利用できる状況にはない。QFT-2GはHIV感染者においてもツ反応に比べより有用な結核感染診断法であることは明らかであり，その特徴を十分理解して適切に用いるべきである。

今回の検討で，HAART施行中の結核既往群では，HAARTによりCD4数が増加し細胞性免疫が回復し，ツ反応が90.9%と高率に陽転化していたが，QFT-2G陽性者は27.3%と少なかった。結核の治療歴がある非HIV感染者におけるQFT-2G陽性率については，当院で行った検討[10]では，結核の治療終了後1年以上経過している患者43例中，QFT-2G陽性20例（46.5%），判定保留9例（20.9%），陰性14例（32.6%）であった。非HIV感染者に比べ，HIV感染者では結核の治療終了後のQFT-2G陽性率はやや低い傾向があった。結核の治療終了後もQFT-2Gが陽性であることの意味付けは難しく，依然として結核菌が存在することを示すのか，免疫の記憶だけが残っているのか議論の多いところである。QFT-2G陽性者についてはQFT-2Gの変動，結核再燃の有無などについて経過を注意深く追う必要がある。

また，HIV感染者における接触者検診や潜在結核感染症の診断にQFT-2Gは有用と考えられる[9]ので，今後のデータの蓄積が必要である。

結　論

（1）HIV感染症合併結核例の13例中1例にQFT-2G判定不可例を認めた。CD4数が著しく低下した症例では判定不可となる可能性がある。

（2）HIV感染症合併結核におけるQFT-2Gの感度は76.9%であり，ツ反応の15.4%よりも有意に高かった。HIV感染症合併結核においてもQFT-2Gは結核感染診断に十分有用であると考えられた。

（3）結核治療終了例のうちQFT-2G陽性者が27.3%あり，この中から結核の再燃が起こるのか注意深い観察が必要と思われた。

〔付記〕

この研究の一部は平成18年度厚生労働科学研究費補助金エイズ対策研究事業「重篤な日和見感染症の早期発見と最適治療に関する研究/主任研究者：安岡　彰（長崎大学医学部歯学部附属病院感染制御教育センター）」の分担研究として行われた。

文　献

1）Mori T, Sakatani M, Yamagishi F, et al.: Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med. 2004 ; 170 : 59–64.

2）The American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC): Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR. 2000 ; 49 (RR-6) : 1–51.

3）Johnson MP, Coberly JS, Clermont HC, et al.: Tuberculin skin test reactivity among adults infected with human immunodeficiency virus. J Infect Dis. 1992 ; 166 : 194–198.

4）Brock I, Ruhwald M, Lundgren B, et al.: Latent tuberculosis in HIV positive, diagnosed by the *M. tuberculosis* specific interferon-gamma test. Respir Res. 2006 ; 7 : 56.

5）Dheda K, Lalvani A, Miller RF, et al.: Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. AIDS. 2005 ; 19 : 2038–2041.

6）Ferrara G, Losi M, D'Amico R, et al.: Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. Lancet. 2006 ; 367 : 1328–1334.

7）Rangaka MX, Wilkinson KA, Seldon R, et al.: The Effect of HIV-1 Infection on T cell Based and Skin Test Detection of Tuberculosis Infection. Am J Respir Crit Care Med. 2006 Dec 7; [Epub ahead of print].

8）Chapman AL, Munkanta M, Wilkinson KA, et al.: Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. AIDS. 2002 ; 16 : 2285–2293.

9）Liebeschuetz S, Bamber S, Ewer K, et al.: Diagnosis of tuberculosis in South African children with a T-cell-based assay : a prospective cohort study. Lancet. 2004 ; 364 : 2196–2203.

10）有賀晴之，川辺芳子，永井英明，他：結核既往者における QuantiFERON-TB 2G testの検討．日本呼吸器学会雑誌．2005 ; 43（増刊）: 154.

Original Article

USEFULNESS OF A WHOLE BLOOD INTERFERON GAMMA ASSAY (QuantiFERON®-TB-2G) FOR DETECTING TUBERCULOSIS INFECTION IN HIV-INFECTED PERSONS

Hideaki NAGAI, Yoshiko KAWABE, Haruyuki ARIGA, Fumiko SHIGIYAMA, Masahiro SHIMADA, Makiko KUNOGI, Yoshinori MATSUI, Masahiro KAWASHIMA, Junko SUZUKI, Nobuharu OOSHIMA, Kimihiko MASUDA, Hirotoshi MATSUI, Atsuhisa TAMURA, Naohiro NAGAYAMA, Shinobu AKAGAWA, Kazuko MACHIDA, Atsuyuki KURASHIMA, and Hideki YOTSUMOTO

Abstract [Background] New blood test (QuantiFERON®-TB-2G: QFT-2G), based on detection of IFN-gamma released by T cells in response to *M. tuberculosis* specific antigens, has the high sensitivity and specificity for diagnosis of tuberculosis. However, it is essential to evaluate this T cell-based approach in individuals with HIV-associated impairment in T cell immunity.

[Methods] We assessed the usefulness of QFT-2G on diagnosis of tuberculosis in 13 HIV-infected patients with tuberculosis and the performance of 25 HIV infected persons under highly active antiretroviral treatment (HAART). QFT-2G, CD4 counts, and tuberculosis skin test and so on were examined.

[Results] The sensitivity of QFT-2G in HIV-infected patients with tuberculosis was 76.9%, which was significantly higher compared with tuberculin skin test, 15.4%. There was one indeterminate case of which CD4 count was 16/μl, the lowest count among the all patients. CD4 counts of 25 HIV infected persons under HAART were between 100 and 1157/μl. There were 3 QFT-2G positive cases among them, who had past history of tuberculosis.

[Conclusion] Although the very low CD4 counts in HIV-infected patients might adversely affect QFT-2G performance, the sensitivity of QFT-2G in the most of HIV-infected patients with tuberculosis was high, and it was thought that it was useful enough to diagnose tuberculosis infection. Careful observation is required in whether the recurrence of tuberculosis takes place among QFT-2G positive persons who have past history of tuberculosis.

Key words: Tuberculosis, HIV infection, QuantiFERON-TB-2G, ESAT-6, CFP-10

Department of Respiratory Diseases, National Hospital Organization Tokyo National Hospital

Correspondence to: Hideaki Nagai, Department of Respiratory Diseases, National Hospital Organization Tokyo National Hospital, 3-1-1, Takeoka, Kiyose-shi, Tokyo 204-8585 Japan. (E-mail: hnagai-in@tokyo-hosp.jp)

MAJOR ARTICLE

Diagnosis of Active Tuberculous Serositis by Antigen-Specific Interferon-γ Response of Cavity Fluid Cells

Haruyuki Ariga,[1] Yoshiko Kawabe,[1] Hideaki Nagai,[1] Atsuyuki Kurashima,[2] Kimihiko Masuda,[1] Hirotoshi Matsui,[1] Atsuhisa Tamura,[1] Naohiro Nagayama,[1] Shinobu Akagawa,[1] Kazuko Machida,[1] Akira Hebisawa,[3] Yutsuki Nakajima,[4] Hideki Yotsumoto,[1] and Toru Mori[5]

Departments of [1]Respiratory Medicine, [2]Clinical Research, [3]Pathology, and [4]Thoracic Surgery, National Hospital Organization, Tokyo National Hospital, and [5]Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

Background. To develop a more accurate methodology for diagnosing active tuberculous pleurisy, as well as peritonitis and pericardits of tuberculous origin, we established an antigen-specific interferon γ (IFN-γ)–based assay that uses cavity fluid specimens.

Methods. Over a 19-month period, 155 consecutive, nonselected patients with any cavity effusion were evaluated. Study subjects were 28 patients with bacteriologically confirmed active tuberculous serositis and 47 patients with definitive nontuberculous etiology. Culture was performed for 18 h with fluid mononuclear cells in the supernatant of the effusion together with saline or *Mycobacterium tuberculosis*–specific antigenic peptides, early secretory antigenic target 6 and culture filtrate protein 10. IFN-γ concentrations in the culture supernatants were measured.

Results. In patients with active tuberculous serositis, antigen-specific IFN-γ responses of cavity fluid samples were significantly higher than those of nontuberculous effusion samples. Area under the receiver operating characteristic (AUROC) curve was significantly greater for cavity fluid IFN-γ response (AUROC curve, 0.996) than for cavity fluid adenosine deaminase and whole-blood IFN-γ responses (AUROC curve, 0.882 and 0.719, respectively; $P = .037$ and $P < .001$, respectively). Although the AUROC curve was greater for cavity fluid IFN-γ response than for background cavity fluid IFN-γ level (AUROC curve, 0.975), the AUROC curves were not statistically significantly different ($P = .74$). However, multivariate logistic regression analysis revealed that cavity fluid IFN-γ responses were significantly associated with the diagnosis, even after adjustment for background IFN-γ level (adjusted odds ratio, 1.21; 95% confidence interval, 1.03–1.42; $P < .001$).

Conclusions. The cavity fluid IFN-γ assay could be a method for accurately and promptly diagnosing active tuberculous serositis.

Tuberculosis is a serious infectious disease that threatens the health of mankind, even in the modern world [1]. Active tuberculous pleuritis, peritonitis, and pericarditis should always be considered as possible causes of cavity fluid in the daily practice of clinical medicine. Their definitive diagnosis is based on the identification of *Mycobacterium tuberculosis* after culture of effusion or tissue biopsy specimens. However, the rate of positive results of such cultures of pleural fluid specimens is not high (<30%) [2]. In addition, because bacteriological results cannot be obtained rapidly, antituberculous agents should, in some cases, be instituted on the basis of clinical speculation without waiting for the definitive diagnosis. Measurement of nonspecific markers, such as adenosine deaminase (ADA) and IFN-γ, in the supernatant of fluid specimens has been used to diagnose pleural tuberculosis [3, 4]. A meta-analysis concluded that maximum joint sensitivity and specificity was 93% for the ADA assay and 96% for the IFN-γ assay [5]. It has been reported that the levels of these markers are also increased in the context of other diseases [6–9], and there is some contention as to the use of these tests [10, 11]. Thus, it would be desirable to develop a more specific diagnostic method for active tuberculous serositis (ATBS).

Active tuberculous pleuritis is thought to be caused by the spread of a small subpleural or hilar lymph node

Received 1 May 2007; accepted 2 August 2007; electronically published 8 November 2007.

Reprints or correspondence: Dr. Haruyuki Ariga, Dept. of Respiratory Medicine, Tokyo National Hospital, 3-1-1 Takeoka, Kiyose, Tokyo 204-8585, Japan (arigah-in@tokyo-hosp.jp).

Clinical Infectious Diseases 2007; 45:1559–67

1058-4838/2007/4512-0005$15.00
DOI: 10.1086/523591

caseous focus of *M. tuberculosis* into the cavity, and T cells that specifically respond to tuberculous antigens and produce IFN-γ are recruited in the pleural cavity of patients with active tuberculous pleuritis [12, 13]. *M. tuberculosis*–specific protein antigens early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are absent from the genomes of all bacille Calmette-Guérin substrains and most nontuberculous mycobacteria, have been identified [14]. A specific method for the detection of tuberculous infection has been developed in which IFN-γ production (the number of IFN-γ–producing cells) is measured after stimulation of peripheral blood with these antigens in vitro [15–19]. We hypothesized that, when cavity fluid mononuclear cells of patients with ATBS were stimulated with the antigenic peptides ESAT-6 or CFP-10, IFN-γ responses that are greater than those achieved using the stimulation of peripheral blood might be expected. We report our results, which suggest the possibility of accurate diagnostic procedures for ATBS that reflect the local immune response in the cavity.

PATIENTS AND METHODS

Patients and evaluation of their cavity effusion specimens. Over a 19-month period from November 2004 through June 2006, 155 consecutive, nonselected patients with any cavity effusion were evaluated at the inpatient department of Tokyo National Hospital (Tokyo, Japan). Either cavity centesis or endoscopic procedures, including thoracoscopic and perioteneoscopic procedures, was performed for all patients; 75 patients for whom the etiology of cavity effusion could be definitively identified were enrolled. Table 1 shows the profiles and the clinical diagnoses that these patients received. All patients with tuberculosis had received <7 days of antituberculous chemotherapy at the time of testing. None of these patients had conditions, such as HIV infection, associated with severe immunocompromise, and none were receiving immunosuppressive drugs.

The differential diagnosis based on the evaluation of the pleural effusion specimens was made using the following criteria. A definitive diagnosis of active tuberculous pleuritis, peritonitis, or pericarditis was made on the basis of positive culture results and identification of *M. tuberculosis* in cavity effusion or parietal tissue specimens. With regard to nontuberculous pleuritis, peritonitis, and pericarditis, the diagnosis of neoplastic pleuritis, peritonitis, or pericarditis was based on detection of malignant cells in pleural effusion or tissue specimens; parapneumonic effusion was defined by the presence of fever, spu-

Table 1. Profiles of patients with cavity effusion.

Variable	Patients with active tuberculous serositis (n = 28)	Patients with nontuberculous effusion (n = 47)	All (n = 75)
Age, years			
Mean ± SD	60.5 ± 22	72.1 ± 10.8	67.9 ± 17.1
Range	20–91	46–91	20–91
Sex			
Male	24 (86)	40 (85)	65 (87)
Female	4 (14)	7 (15)	10 (13)
Clinical diagnosis			
Active tuberculous pleuritis	26	0	...
Active tuberculous peritonitis	1	0	...
Active tuberculous pericarditis	1	0	...
Nontuberculous pleuritis	0	45	...
Neoplastic pleuritis	0	24	...
Lung cancer	0	22	...
Malignant mesothelioma	0	2	...
Parapneumonic effusion	0	12	...
Heart failure	0	5	...
Asbestos pleural effusion	0	1	...
Posttraumatic pleural effusion	0	1	...
Mycobacterium avium pleuritis	0	1	...
Hypothyroidism (pleural effusion)	0	1	...
Cancerous peritonitis	0	1	...
Cancerous pericarditis	0	1	...

NOTE. Data are no. or no. (%) of patients, unless otherwise indicated.

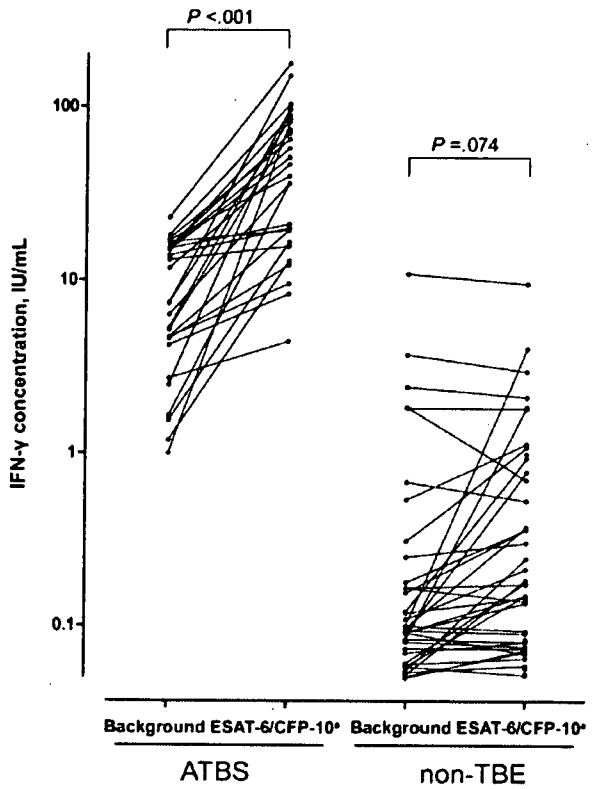

Figure 1. IFN-γ production in response to stimulation with *Mycobacterium tuberculosis*–specific antigen. Pleural, peritoneal, and pericardial fluid cells obtained from patients with active tuberculous serositis (ATBS) or nontuberculous effusion (non-TBE) were harvested and suspended in the individual supernatant of the effusion at 1–5 million cells/mL, stimulated with saline or *M. tuberculosis*–specific antigens, and incubated for 18 h, after which IFN-γ concentrations in the culture supernatant were measured by ELISA. If, in an initial assay, a supernatant was found to contain >15 IU/mL of IFN-γ, the specimen was diluted with zero standard and reassayed. IFN-γ concentrations in each patient, which were expressed as background saline control IFN-γ concentrations and concentrations after stimulation with specific antigens (early secretory antigenic target 6 [ESAT-6] and culture filtrate protein 10 [CFP-10]), are shown as closed circles connected with lines. Student's *t* test was used for differences between the ATBS and non-TBE groups. IFN-γ values after antigen stimulation are the measured values, not values subtracted from the corresponding background control values. [a]The higher IFN-γ concentrations after stimulation with either antigenic peptides ESAT-6 or CFP-10 are represented.

tum, infiltrates on chest radiograph, an infectious inflammatory response detected by serological testing, and improvement after the administration of antibiotics. Heart failure was assigned as the cause of pleural effusion if cardiac dysfunction was evident on ultrasonographic cardiograph and improvement was noted after the administration of diuretics or cardiotonic drugs. Asbestos pleural effusion was diagnosed on the basis of detection of asbestos particles or fibrous patches in pleural biopsy specimens. Posttraumatic hemothorax was indicated when there was evident pooling of blood in the thorax after chest trauma. Pleuritis due to *Mycobacterium avium* was considered on the basis of identification of *M. avium* in pleural effusion specimens.

Stimulation of cavity fluid cells with* M. tuberculosis*–specific antigens (cavity fluid IFN-γ assay). Twenty to 50 mL of cavity effusion were centrifuged at 2000 rpm for 15 min. The supernatant was separated, and the number of nucleated cells in the cell compartment was determined. Cells were then suspended at 1–5 million cells/mL in the individual supernatant of the effusion. One milliliter of this cell suspension was then added to 4 wells of 24-well plates (NUNC) and underwent culture in the presence of *M. tuberculosis* antigen peptides ESAT-6 or CFP-10 at a final concentration of 1 μg/mL (QuantiFERON-TB 2G; Cellestis) [19]. To the remaining 2 wells, physiological saline was added instead of the antigens, because a measurement of background IFN-γ of cavity fluid and phytohemagglutinin (5 μg/mL) was used as a positive control. Cells were incubated at 37°C for 18 h in a 5% CO_2 incubator, and the cultured supernatant was harvested for measurement of IFN-γ concentration. The value of the cavity fluid assay was defined as the difference between the determined higher value after stimulation with either ESAT-6 or CFP-10 and the value of the measurement of background IFN-γ. The whole-blood IFN-γ assay was performed as previously described [19].

Measurement of IFN-γ and ADA concentrations. IFN-γ concentration was measured using the QuantiFERON-CMI ELISA (Cellestis) according to procedures described in the manufacturer's instructions. The IFN-γ values were expediently replaced with 0.05 when the difference between the antigen-stimulated level and the background level was negative or <0.05 IU/mL (lower limit of measurement). If, in an initial assay, the supernatant was found to contain >15 IU/mL of IFN-γ (upper limit of measurement), the specimen was diluted with zero standard and reassayed. When both stimulated and background IFN-γ concentrations were extremely high, we performed another culture using plasma samples from each patient for medium to decrease the background IFN-γ concentration. The result of the whole-blood assay was considered to be indeterminate if the value for the positive control sample was <0.5 IU/mL after subtraction of the value for the negative control sample. ADA concentration in the supernatant of cavity fluid samples was measured by AUTO A MIZUHO ADA (Mizuho Medy). Three technicians with expertise in laboratory work were blinded to the clinical information of the patients, results of other assays in this study, and the reference standard.

Statistical analysis. Univariate analyses were performed using Student's *t* test and the Mann-Whitney *U* test. We constructed a receiver operating characteristic (ROC) curve by

Table 2. IFN-γ response in cavity fluid and whole-blood cells to tuberculous-specific and nonspecific antigens and adenosine deaminase (ADA) levels in patients with active tuberculous serositis and patients with nontuberculous effusion.

Variables	Patients with active tuberculous serositis (n = 28)	Patients with nontuberculous effusion (n = 47)	P
Background IFN-γ level in cavity fluid	7.4 (4.5–15.2)	0.09 (0.05–0.16)	<.001
ESAT-6–background[a]	26.4 (6.8–49.4)	0.05 (0.05–0.08)	<.001
CFP-10–background[b]	12.2 (2.8–37.7)	0.05 (0.05–0.09)	<.001
ESAT-6 or CFP-10–background[c]	34.8 (7.3–74.3)	0.05 (0.05–0.19)	<.001
Mitogen–background[d]	22.6 (2.1–55.1)	6.90 (0.32–21.5)	.009
Whole-blood IFN-γ assay result[e]	0.54 (0.3–0.9)	0.07 (0.05–0.54)	<.001
ADA concentration[f]	61.3 (43.0–71.5)	13.80 (7.95–27.5)	<.001

NOTE. Data are median value (interquartile range). Mann-Whitney U test was used for differences in IFN-γ and ADA concentrations between the patients with active tuberculous serositis and patients with nontuberculous effusion. CFP-10, culture filtrate protein 10; ESAT-6, early secretory antigenic target 6.

[a] Difference between the determined IFN-γ concentration after stimulation with ESAT-6 and the background IFN-γ concentration in cavity fluid.

[b] Difference between the determined IFN-γ concentration after stimulation with CFP-10 and the background IFN-γ concentration in cavity fluid.

[c] Difference between the determined higher IFN-γ concentration after stimulation with either ESAT-6 or CFP-10 and the background IFN-γ concentration.

[d] Difference between the determined IFN-γ concentration after stimulation with mitogen and the background IFN-γ concentration in cavity fluid.

[e] None of the patients had indeterminate results, and 1 patient with tuberculosis was unavailable.

[f] Not performed for 1 patient with tuberculosis.

plotting the rate of sensitivity against the rate of false-positive results over a range of cutoff values of IFN-γ and ADA for the assessment of diagnostic accuracy. Area under the ROC curve was calculated using the trapezoidal rule. Standard errors of the areas and comparisons between assays were calculated using a method described elsewhere [20]. Pairwise comparisons were made between area under the ROC curve for the cavity fluid IFN-γ assay and that for each of the following measures: background IFN-γ and ADA assays and the whole-blood IFN-γ assay. Adjustment for multiple comparisons was made using the Dunn-Sidák method. To assess whether the cavity fluid assay lends additional diagnostic information to that provided by the background IFN-γ level, these 2 measurements were introduced as predictors in a multivariate logistic regression model. These 2 explanatory variables were treated as continuous variables. $P < .05$ was considered to be statistically significant. All statistical analyses were performed using Stata, version 9 (Stata).

This study was approved by the ethics review committee of our hospital. We obtained informed consent from all participants in the study.

RESULTS

The mean age of patients with nontuberculous effusion (non-TBE) was significantly higher than that of patients with ATBS (72.4 years vs. 60.5 years; $P = .013$). There was no statistically significant difference in sex between the groups (85% male vs. 81% male; $P = .745$) (table 1).

Figure 1 shows results of the cavity fluid IFN-γ assay for each subject (in both the ATBS and non-TBE groups), including background IFN-γ values and the highest values for either of the stimulation antigens, ESAT-6 or CFP-10. The IFN-γ concentration was significantly increased after antigen stimulation, compared with the background IFN-γ concentration, in every patient with ATBS ($P < .001$). However, in patients with non-TBE, IFN-γ concentrations did not significantly increase after stimulation with antigens ($P = .074$)

The median values and the interquartile range of background cavity fluid IFN-γ, antigen-specific cavity fluid IFN-γ, whole-blood, and ADA assays are shown in table 2. Individual values for these parameters, as well as the result of the cavity fluid assay multiplied by the background IFN-γ level, are indicated by dots in figure 2. Background IFN-γ concentrations were significantly higher in patients with ATBS than in patients with non-TBE. Cavity fluid IFN-γ responses to both ESAT-6 and CFP-10 were significantly greater for patients with ATBS than for patients with non-TBE. Likewise, when an IFN-γ response was represented by a higher value of response to either ESAT-6 or CFP-10, the difference between the values for the ATBS group and the non-TBE group was greater. IFN-γ responses to mitogen were significantly greater for patients with ATBS than