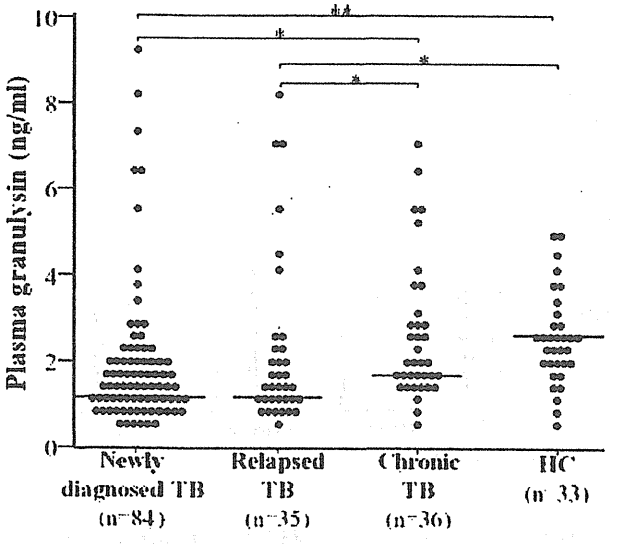

Fig. 1. Circulating granulysin concentrations in patients with newly diagnosed, relapsed and chronic TB in comparison with healthy controls. Each dot represented one individual. The horizontal bars indicate the median of each group. *, $P < 0.05$; **, $P < 0.001$; HC, healthy control.

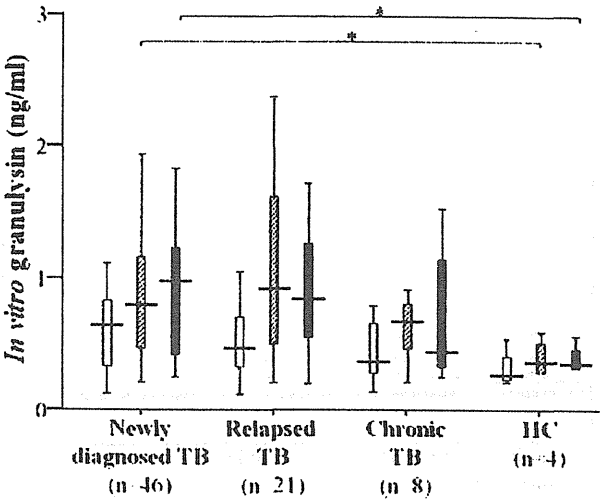

Fig. 2. *In vitro* granulysin production by PBMCs from patients with newly diagnosed, relapsed and chronic TB and healthy individuals stimulated with PPD (diagonal shading) and heat killed *Mycobacterium tuberculosis* (H37Ra) (black). Supernatant from PBMCs without stimulation was used as controls (clear). The horizontal bars indicate the median of each group. *, $P < 0.05$.

healthy controls stimulated *in vitro* with PPD (median ± SE = 0.359 ± 0.073 ng/mL, range 0.283–0.591 ng/mL), and H37Ra (median ± SE = 0.348 ± 0.056 ng/mL, range 0.320–0.559 ng/mL) ($P = 0.022$, $r = -2.289$ and $P = 0.032$, $r = -2.146$, respectively). Controls were PBMC supernatants from healthy controls without stimulation (median ± SE = 0.262 ± 0.076 ng/mL, range 0.206–0.542 ng/mL) and PBMC supernatants from newly diagnosed TB patients without stimulation (median ± SE = 0.636 ± 0.051 ng/mL, ranged 0.117–1.665 ng/mL). Although granulysin production by relapsed TB-PBMCs stimulated *in vitro* with PPD (median ± SE = 0.922 ± 0.146 ng/mL, range 0.205–2.374 ng/mL) and H37Ra (median ± SE = 0.841 ± 0.123 ng/mL, range 0.197–2.324 ng/mL) were higher than those of healthy controls, these differences were not significant ($P = 0.054$, $r = -1.927$ and $P = 0.081$, $r = -1.742$, respectively). PBMCs of patients with chronic TB stimulated *in vitro* with PPD (median ± SE = 0.674 ± 0.120 ng/mL, range 0.475–1.345 ng/mL) and H37Ra (median ± SE = 0.435 ± 0.173 ng/mL, range 0.408–1.521 ng/mL) produced greater amounts of granulysin than did healthy controls, the difference not being significant ($P = 0.089$, $r = -1.698$ and $P = 0.497$, $r = -0.679$, respectively). Similar median amounts of granulysin were produced by PBMCs of newly diagnosed and relapsed TB stimulated *in vitro* with PPD and H37Ra but higher amounts by PBMCs of chronic TB, the difference not being significant (newly diagnosed and chronic TB: $P = 0.330$, $r = -0.974$ for PPD and $P = 0.242$, $r = -1.169$ for H37Ra; relapsed and chronic TB: $P = 0.232$, $r = -1.196$ for PPD and $P = 0.380$, $r = -0.878$ for H37Ra) (Fig. 2).

Circulating interferon-γ concentrations in clinical tuberculosis before anti-TB therapy

In contrast to granulysin, the circulating IFN-γ concentrations in patients with newly diagnosed TB (median ± SE = 6.15 ± 4.58 pg/mL, range < 4.7–300 pg/mL) and relapsed TB (median ± SE = 7.93 ± 8.86 pg/mL, range <4.7–310.73 pg/mL) were significantly higher than those of healthy controls (median ± SE = <4.7 ± 0.20 pg/mL, range <4.7–10.13 pg/mL) ($P < 0.001$, $r = -3.923$ and $P < 0.001$, $r = -4.325$, respectively). Circulating IFN-γ concentrations in most chronic TB patients were similar to those of healthy individuals (median ± SE = <4.7 ± 3.76 pg/mL, range <4.7–123.69 pg/mL) ($P = 0.051$, $r = -3.486$). The median concentrations of IFN-γ were similar in patients with newly diagnosed and relapsed TB, but both were higher than in chronic TB, the difference not being significant ($P = 0.395$, $r = -0.851$ and $P = 0.333$, $r = -0.968$, respectively) (Fig. 3).

Interferon-γ production in peripheral blood mononuclear cell stimulation assay

The median IFN-γ production by PBMCs of newly diagnosed TB patients stimulated *in vitro* with PPD (median ± SE = 535 ± 94 pg/mL, range <4.7–2400 pg/mL) was higher than that of healthy controls (median ± SE = 434 ±

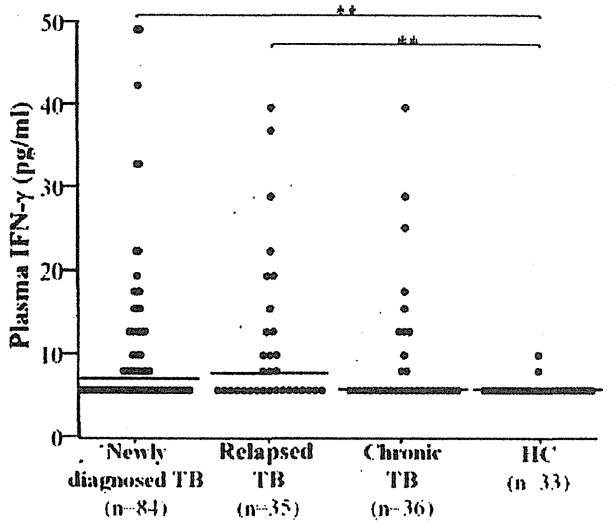

Fig. 3. Circulating IFN-γ concentrations in patients with newly diagnosed, relapsed and chronic TB in comparison with healthy controls. Each dot represents one individual. The horizontal bars indicate the median of each group. **, $P < 0.001$; HC, healthy control.

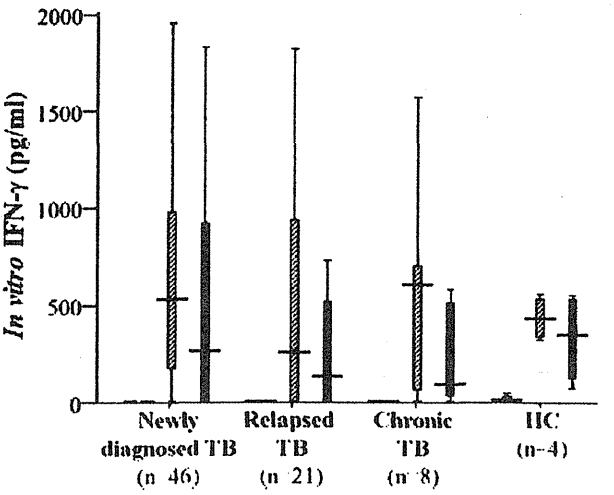

Fig. 4. *In vitro* IFN-γ production by PBMCs from patients with newly diagnosed, relapsed and chronic TB and healthy individuals stimulated with PPD (diagonal shading) and H37Ra (black). Supernatant from PBMCs without stimulation was used as controls (clear). The horizontal bars indicate the median of each group.

57 pg/mL, range 326–562 pg/mL) ($P = 0.591$, $r = -0.537$). However, most newly diagnosed TB-PBMCs stimulated *in vitro* with H37Ra produced higher IFN-γ concentrations (range <4.7–8025 pg/mL), but the median was similar (median ± SE = 270 ± 260 pg/mL) to that of healthy controls (median ± SE = 351 ± 120 pg/mL, range 76–556 pg/mL) ($P = 0.914$, $r = -0.107$). Supernatant from PBMCs without stimulation was used as a cell control (median ± SE = 14.29 ± 8.88 pg/mL, range 9.85–48.06 pg/mL), while supernatant from newly diagnosed TB-PBMCs without stimulation was used as a control for IFN-γ production (median ± SE = <4.7 ± 5.08 pg/mL, range <4.7–231 pg/mL). IFN-γ production by PBMCs from half the patients with relapsed TB stimulated either with PPD (range <4.7–4225 pg/mL) or H37Ra (range <4.7–2575 pg/mL) was higher than that of normal controls. However, their medians (median ± SE = 260 ± 258 pg/mL for PPD, and median ± SE = 138 ± 136 pg/mL for H37Ra) were lower than those of healthy controls; these differences were not significant ($P = 0.823$, $r = -0.223$ and $P = 0.412$, $r = -0.821$, respectively). Chronic TB-PBMCs stimulated *in vitro* with PPD (median ± SE = 610 ± 166 pg/mL, range <4.7–1575 pg/mL) produced higher IFN-γ concentrations than did healthy controls, and some PBMCs stimulated *in vitro* with H37Ra also produced higher IFN-γ concentrations (range <4.7–1835 pg/mL) although the median was lower (median ± SE = 95 ± 198 pg/mL) than that of healthy controls ($P = 0.758$, $r = -0.309$ and $P = 0.354$, $r = -0.927$, respectively). Similar median amounts of IFN-γ production by PBMCs of newly diagnosed and chronic TB stimulated *in vitro* with PPD were found, and these were higher than for relapsed TB, the difference not being significant ($P = 0.436$, $r = -0.779$ and $P = 0.928$, $r = -0.091$, respectively). The median amount of IFN-γ produced by PBMCs of newly diagnosed TB stimulated *in vitro* with H37Ra was higher than that for relapsed and chronic TB ($P = 0.202$, $r = -1.275$ and $P = 0.982$, $r = -0.023$, respectively) (Fig. 4).

DISCUSSION

In this study, the correlations of plasma granulysin and IFN-γ concentrations with clinical disease in patients with newly diagnosed pulmonary, relapsed and chronic TB in northern Thailand, where TB is endemic, were evaluated. The effects of *in vitro* stimulation with PPD and H37Ra of PBMCs from these patients were also investigated. The finding of decreased circulating granulysin and increased IFN-γ in patients with newly diagnosed, relapsed and chronic TB before anti-TB therapy indicated involvement of granulysin and IFN-γ in host defense against TB infections.

In patients with newly diagnosed and relapsed pulmonary TB who had not yet received anti-TB therapy, plasma granulysin concentrations were significantly decreased compared to those of healthy individuals. This may be because granulysin is rapidly consumed during active disease, because of an ongoing effector immune response, or because plasma granulysin is reduced during active disease because of a reduction in the T cell subset dedicated to its production (15). However, granulysin

concentrations in patients with chronic TB, which had not been eradicated by treatment with conventional anti-TB drugs, and who had persistent clinical symptoms and progression of disease, were also lower than in healthy individuals. It is possible that persistence of clinical disease is associated with deficient expression of perforin and granulysin at the local site of TB infection (16). Although significant infiltration of T cells (CD3+, CD4+ and CD8+ T cells) is evident in TB lesions in patients with persistent inflammation, there are only small amounts of perforin and granulysin in these lesions, and evidence of severely impaired expression of these cytolytic effector molecules inside the distinct granules (16). Simultaneously, the numbers of granzyme A-expressing cells are increased in TB lesions, suggesting that the down-regulation of perforin and granulysin is selective and not a universal phenomenon involving all cytolytic effector molecules. These results are similar to those of recent studies which demonstrated that circulating granulysin reaches concentrations similar to those of healthy controls during TB therapy and increases further after completion of therapy (14, 15). However, larger sample sizes are necessary to gain better insight into the dynamics of plasma granulysin concentrations.

In contrast to granulysin, the concentrations of circulating IFN-γ in patients with newly diagnosed and relapsed TB were significantly higher than those of healthy controls, suggesting that IFN-γ plays a role in the regulatory and effector phases of the immune response to *Mtb* infection. In general, IFN-γ is synthesized from CD4+T cells that have been activated by recognition of mycobacterial antigen on APCs (9), as well as by CD8+ T cells from both mice and humans specific for mycobacterial antigens (17).

However, when recurrent TB was analyzed in this study, including both relapsed and chronic TB, granulysin concentrations were found to be significantly lower ($P = 0.038$, $r = -2.071$), whereas IFN-γ concentrations were significantly higher, than in controls ($P < 0.001$, $r = -4.180$, respectively), the concentrations being similar to those found in newly diagnosed TB, which is possibly due to patients with recurrent TB becoming as active as those with newly diagnosed TB. In this study, the proportional decrease in granulysin and increase in IFN-γ concentrations in newly diagnosed TB was not significantly different from that found in relapsed TB. Possible explanations are that: (i) both types of TB were active at the time of enrollment; and (ii) patients with relapsed TB had lost their immunity to *Mtb* and become active in the same way as newly diagnosed TB (because the relapsed TB patients had previous histories of newly diagnosed TB [their first episodes], re-exposure [second episode] and were registered as relapsed TB on enrollment in this study with a duration of 1–180 months [median 12 months]) between their initial treatment success and diagnosis of relapse. It is not possible to ascertain whether the episodes of relapse represented reactivation of previously inadequately treated TB, or reinfection with a new *Mtb* strain. The present results are similar to previous findings that plasma IFN-γ concentrations are significantly higher in patients with active pulmonary TB than in healthy controls and decrease after treatment. These findings might be because circulating IFN-γ comes from both local production and spill-over of IFN-γ from activated lymphocytes sequestered at the site of *Mtb* infection, as previously described (9, 14, 18). In chronic TB, circulating IFN-γ concentrations did not increase in most patients. Clearly, substantial CD4+ T cell responses occur in patients infected with *Mtb*. Failure of that response to eliminate bacteria may be partially at the level of recognition and activation of infected macrophages. *Mtb* is known to be equipped with numerous immune evasion strategies, including modulation of antigen presentation to avoid elimination by T cells. There is evidence that *Mtb*-infected macrophages have diminished ability to present antigens to CD4+ T cells, apart from IFN-γ production, which would contribute to the inability of the host to eliminate persistent infection (19).

In contrast, when PBMCs from newly diagnosed, relapsed and chronic TB were stimulated *in vitro* with PPD or H37Ra, they produced more granulysin than did stimulated controls, a finding which is in contrast to the median and individual concentrations of circulating granulysin. Possible explanations for this discrepancy are that: (i) during *in vivo* stimulation during active disease, granulysin might be rapidly consumed because of the ongoing effector immune response; (ii) *in vivo* serum granulysin is reduced during active disease because of a reduction in the T cell subset dedicated to its production (15); or (iii) when PBMCs that possibly contain primed T cells (indicated by high plasma concentrations of granulysin) are re-stimulated *in vitro* with either PPD and H37Ra, they may produce more granulysin in the supernatant. A related phenomenon has been reported in which stimulation with PPD *in vitro* PBMCs from healthy tuberculin skin test positive individuals results in increased granulysin expression in PPD-stimulated CD4+ and CD8+ T cells, compared to that of unstimulated cells (20). Moreover, it has been reported that, after stimulation *in vitro* with *Mtb* including H37Ra, both CD4+ and CD8+ T cells up-regulate mRNA expression for granulysin, granzyme A and B, perforin and CD95L (Fas ligand), and are able to lyse *Mtb* infected target cells, this being mediated primarily through the granule exocytosis pathway (21).

Median and individual concentrations of circulating IFN-γ in patients with newly diagnosed and relapsed TB were significantly higher than in healthy controls. Similar

results, namely greater IFN-γ production than in stimulated healthy controls, were seen with *in vitro* stimulation with PPD and H37Ra of PBMCs from most patients with newly diagnosed and half of relapsed TB patients, although some stimulated PBMCs from these patients produced less IFN-γ. However, the median IFN-γ production with *in vitro* stimulation of PBMCs from relapsed TB patients is lower than that of healthy controls. Surprisingly, PBMCs from healthy individuals stimulated *in vitro* with PPD and H37Ra in this study did induce significant IFN-γ production. However, these four healthy individuals were recruited from the Blood Bank of a provincial hospital in Chiang Rai where TB is endemic, and did not undergo chest X-ray, TST and any testing for latent TB infection and infection manifesting as active TB by IGRAs. At the time of recruitment, based on their histories, these individuals were thought to be healthy blood donors. However, we cannot be sure that they had never been exposed to *Mtb* and remained asymptomatic, or been vaccinated with BCG. It is known that 5–10% of those infected with *Mtb* will progress towards active TB during their lifetime, whereas the remainder are resistant to active TB, but remain infected. In fact, most Thai people are vaccinated with BCG since child. Therefore, it is possible that these healthy individuals had been exposed to *Mtb* in their lifetime, and that this had caused the high production of IFN-γ after stimulation *in vitro* with PPD and H37Ra. More normal healthy individuals from non-endemic TB areas who have been confirmed negative by chest X-ray and TST, and tested for latent TB infection and infection manifesting as active TB by IGRAs, should be included in future studies.

IFN-γ is produced from T cells (both CD4+ and CD8+T cells) and NK cells and activates bactericidal mechanisms in macrophages (3). It has been demonstrated that during the course of chronic and fatal TB infection, CD4+ T cells are absent even though CD8+ T cells can produce large amounts of IFN-γ. This supports the hypotheses that CD4+ T cells have important, non redundant roles in control of *Mtb* in addition to IFN-γ production, that CD4+ T cells assist in the development of cytotoxic CD8+ T cell populations and that the cytotoxicity exerted by effector CD8+ T cells might be an important component of anti-mycobacterial immunity (22). The present results indicate that patients with newly diagnosed and relapsed TB have low circulating granulysin but high IFN-γ concentrations before anti-TB therapy, suggesting that granulysin and IFN-γ may act in concert or in synergy in host defense against *Mtb* infection.

In conclusion, patients with active pulmonary TB have low circulating granulysin but high IFN-γ concentrations before treatment indicating their possible role in controlling *M. tuberculosis* infection.

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Elevated anti-tuberculous glycolipid antibody titres in healthy adults and tuberculosis patients in Thailand

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SUMMARY

OBJECTIVE: To evaluate immunoglobulin G (IgG) and immunoglobulin A (IgA) responses to tuberculous-glycolipid antigen (TBGL-IgG and -IgA) in pulmonary tuberculosis (TB) patients and healthy controls in Thailand.

DESIGN: Anti-TBGL antibody titres and other TB related markers were measured in the serum samples of 24 adults with pulmonary TB (PTB), 28 healthy adults (HA), 23 children with TB and 24 healthy children.

RESULT: Both TBGL-IgG and -IgA titres were significantly higher only in adult PTB cases compared to controls ($P < 0.001$ for all). TBGL-IgG was highly sensitive (92%) in PTB patients, but frequent positive proportions of TBGL-IgG (46%) and -IgA (36%) in HAs were the cause of low specificities of TBGL-IgG (54%) and -IgA (64%); that of TBGL-IgG+IgA (75%) was the highest. Antibody titres were positively correlated in TBGL-IgG+IgA double-positive HAs (HA+, 7/28, $P < 0.01$), but not in HA− ($P > 0.05$). Serum IgG and IgA levels were not correlated with TBGL-IgG or -IgA levels ($P > 0.05$). KL-6 and leptin levels were normal and were not different between HA+ and HA−, indicating absence of active TB in HAs.

CONCLUSION: Enhanced TBGL-IgG+IgA responses in HAs could indicate latent TB infection. Careful follow-up studies in HAs could clarify the significance of elevated TBGL antibodies as early disease markers.

KEY WORDS: anti-tuberculosis glycolipid IgG; TBGL; IgA; TB-endemic country; latent TB infection

MYCOBACTERIUM TUBERCULOSIS is a leading global health problem that caused an estimated 9.27 million new cases of tuberculosis (TB) infection and more than 2 million deaths worldwide in 2007.[1] The alarming increase in the incidence of multidrug-resistant TB, particularly among human immunodeficiency virus (HIV) infected patients,[1] and the development of the immune reconstitution syndrome after the initiation of highly active antiretroviral treatment (HAART),[2] have rendered the situation more critical. Conventional microscopy, which has a variable range of sensitivity of 20–60% in detecting tubercle bacilli, is widely used by resource-limited countries,[3] which harbour more than 90% of the world's TB infection.[1] However, approximately 20% of TB cases are not microbiologically proven, even with the more expensive fluorescence microscopy.[3,4] Moreover, a bacteriologically confirmed diagnosis of TB in paediatric groups is much more difficult, as children seldom produce sputum. There is therefore an urgent need to develop an early diagnostic approach to identify both paediatric and adult TB patients.

Cord factor (trehalose-6-6-dimycolate; TDM), which composes a major part of the mycobacterial cell wall, has been identified as the most immunogenic glycolipid; it is produced mainly by virulent *M. tuberculosis* as well as by atypical mycobacteria.[5,6] Tuberculous-glycolipid antigen (TBGL) consists of purified TDM from H37Rv.[7] The immunoglobulin G (IgG) response to TBGL antigen (TBGL-IgG) has been proposed as a useful tool for TB serodiagnosis (sensitivity and specificity >80%) in Japan, a non-TB-endemic country (incidence rate 20 per 100 000 population).[7,8] Although IgG and immunoglobulin A (IgA) responses to purified TB antigens and a commercial serological assay were demonstrated to have limited significance for the serodiagnosis of pulmonary tuberculosis (PTB) in a meta-analysis and systemic review by Steingart et al., of the lipid antigens, cord factor showed particularly high reactivity.[9,10] IgA responses against the mycobacterial fusion protein MT10.3: MPT64 was recently demonstrated to have higher sensitivity for the diagnosis of extra-pulmonary TB in a TB-endemic country.[11] Although the diagnostic efficacy

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of TBGL-IgA was not evaluated in prior studies, a significant association between TBGL-IgG and -IgA was reported in active TB patients.[12] However, IgG or IgA responses against TBGL antigen have not been evaluated for their diagnostic ability in TB-endemic countries.

As TBGL-IgG titres were found to be associated with C-reactive protein and cavity formation,[12] other markers related to TB pathology, including KL-6 and leptin, could have some role in promoting inflammation in PTB. A high-molecular-weight mucinous glycoprotein expressed on type-II pneumocytes, KL-6 was reported to be elevated in the serum of patients with interstitial pneumonia[13] and PTB with extensive radiographic changes.[14] Leptin, a cytokine-like hormone produced by the bronchial epithelial cells and type-II pneumocytes in addition to adipose tissue,[15] was reported to be low in the serum of PTB patients.[16]

The purpose of the present study was to evaluate IgG and IgA antibody (Ab) responses to the TBGL antigen in adult and children TB patients and healthy controls in Thailand. The relationship of TBGL antibodies to KL-6 and leptin was also assessed.

MATERIALS AND METHODS

Subjects

A case-control study was conducted between April 2007 and October 2008. Adult cases (age >16 years) were 24 newly diagnosed active PTB patients receiving care at the Chest Disease Institute (CDI), Nonthaburi, who were enrolled before or within 2 weeks of receiving anti-tuberculosis treatment. All of the PTB patients were positive for sputum acid-fast bacilli (AFB) stain and culture for *M. tuberculosis*.

Twenty-three children (age ≤12 years) diagnosed with TB and receiving care at the Queen Sirikrit National Institute of Child Health (QSNICH), Bangkok, were enrolled as child TB cases (CTB) before receiving anti-tuberculosis treatment. They were diagnosed with active TB based on the presence of two or more features suggestive of probable TB, including history of close TB contact, positive tuberculin skin test (TST) response (>10 mm diameter), chest X-ray (CXR) findings suggestive of TB, and histopathological features related to TB according to the diagnostic criteria of the World Health Organization (WHO) provisional guidelines for the diagnosis of paediatric TB.[17] Diagnosis was confirmed by positive culture of tubercle bacilli.

Subjects with underlying malignancy, metabolic disorders, HIV/AIDS (acquired immune-deficiency syndrome) or other active pulmonary diseases were excluded from the study.

Healthy adult individuals with no concomitant pulmonary symptoms, normal CXR and negative HIV serology were recruited from among blood donor subjects as healthy adult controls (HA). Volunteer healthy child controls (HC) were selected from among paediatric patients without respiratory symptoms and with normal CXR from the surgical department of the QSNICH.

Blood samples were collected from all enrolled participants. Serum samples were separated and stored in −20°C for further study.

This study was approved by the ethics committees of all the participating institutes in Thailand and Japan. Written informed consent was obtained from all enrolled participants. The study was conducted according to the recommendations of the Helsinki Declaration.

TBGL antibodies

TBGL-IgG and -IgA titres were measured using the Determiner TBGL-antibody ELISA kit (Kyowa Medex, Tokyo, Japan), an in vitro enzyme-linked immunosorbent assay (ELISA) kit for the quantitative measurement of TBGL-IgG and -IgA in serum or plasma. Antibody titres for both antibodies were expressed as U/ml. Samples were classified as TBGL-IgG-positive if TBGL-IgG serum levels were ≥2 U/ml.[7] An arbitrary cut-off value of ≥2 U/ml for TBGL-IgA was used as per the unpublished data of our previous study.[12]

ELISA assay

Serum leptin and sIL-2Rα levels were determined by sandwich ELISA using the Quantikine Human Leptin Immunoassay kit and the Quantikine Human IL-2 sRα Immunoassay kit (both from R&D Systems, Minneapolis, MN, USA) for the quantitative determination of the human leptin and sIL-2Rα concentrations respectively in serum or plasma according to the manufacturer's guidelines. Serum KL-6 levels were measured using an ELISA kit (Sanko-junyaku, Tokyo, Japan).

Measured laboratory markers

We assessed the whole blood profile as well as the serum levels of IgG and IgA and hepatic enzymes (aspertate amino-transferase [AST] and alanine amino-transferase [ALT]).

Statistical analysis

Data were analysed using Statcel 2 (OMS Publishing Inc, Saitama, Japan). We compared sensitivity and specificity using the χ^2 test for proportions. Values are presented as median and range. Differences in titres of different variables between two groups were analysed using the Mann-Whitney *U*-test. Correlations between each variable were evaluated using Spearman's rank correlation coefficient. A two-tailed $P < 0.05$ was considered significant.

RESULTS

Subjects

The demographic and clinical characteristics of the enrolled case participants are shown in Table 1.

Table 1 Demographic and clinical characteristics of study participants

Variable	Adult PTB cases (n = 24) n (%)	Healthy adults (n = 28) n (%)	Child TB patients (n = 23) n (%)	Healthy child controls (n = 24) n (%)
Male:female*	23:1	19:9	12:11	19:9
Age, years, median [range]	36.5 [20–50]	35.5 [21–52]	2 [0.5–12]	3.5 [0.6–12]
TST responses (>10 mm/<10 mm/0–5 mm)	ND	ND	19/1/3	ND
Sputum AFB stain and culture positive	24 (100)	ND	1 (4)	ND
Chest X-ray				
Normal	—	28 (100)	—	24 (100)
Pulmonary infiltration	8 (33.3)	—	11 (47)	—
Infiltration+fibrosis	1 (4.1)	—		—
Miliary infiltration		—	2 (8.6)	—
Hilar lymphadenopathy		—	9 (39)	—
Consolidation/cavity/calcification	1/1/1 (4 in each)	—	0/0/3 (13)	—
Diagnosis		—		—
PTB	24 (100)	—	21 (91)	—
EPTB		—	2 (9)	—

*Frequency.
PTB = pulmonary tuberculosis; TST = tuberculin skin test; ND = not done; AFB = acid-fast bacilli; EPTB = extra-pulmonary TB.

Among the 58 adult participants screened, 24 microbiologically confirmed PTB cases with male predominance (96%) and 28 age-matched HA subjects (male 68%) were included in the analysis; six PTB cases were eventually excluded due to HIV co-infection. In contrast, *M. tuberculosis* infection was not confirmed in 23 CTB cases except one; 19 (83%) children had positive TST responses (>10 mm diameter), including 12 who had a history of TB contact through family members. Although the TST response was <10 mm (range 0–10 mm) in the other four cases, they also had a history of TB contact. On CXR, 21 had pulmonary infiltration and/or hilar lymphadenopathy and other abnormalities relevant to PTB. Two others had massive pleural effusion and features of non-necrotising granulomatous pruritis suggestive of extra-pulmonary TB. Twenty-four age-matched children with no TB-related symptoms and normal CXR findings were enlisted for analysis as controls (HC).

Anti-TBGL antibodies and their correlations

In the adult participants, the TBGL-IgG and -IgA titres were elevated in respectively 22/24 (92%) and 17/24 (63%) PTB cases and 13/28 (46%) and 10/28 (36%) HAs. TBGL-IgG and -IgA titres were significantly higher in the PTB group than in the controls (P < 0.001 for both; Figure 1A, Table 2). The sensitivities of the TBGL-IgG and -IgA assay were 92% and 63%

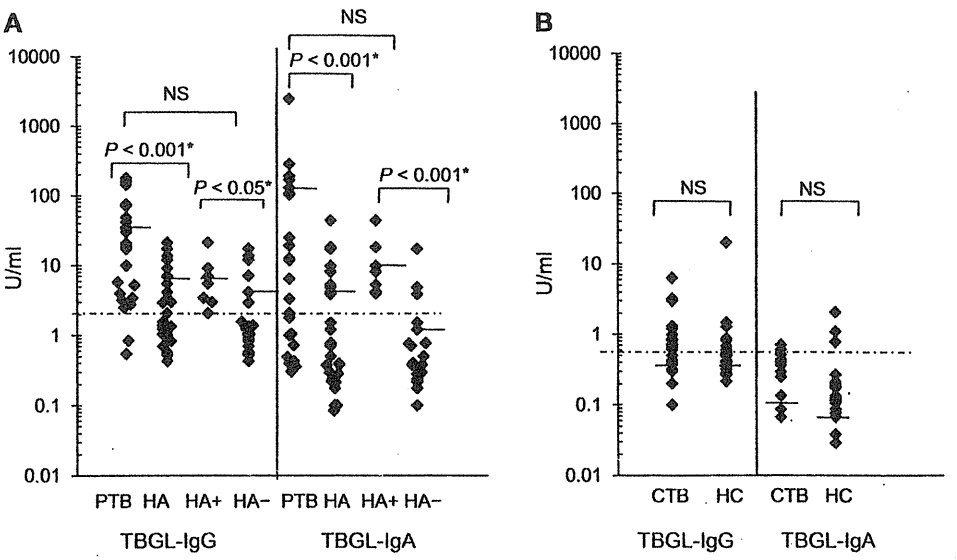

Figure 1 TBGL-IgG and TBGL-IgA titres in **A)** adult and **B)** child participants. Dashed lines indicate the cut-off value of ≥2 U/ml for both antibodies. Solid bars indicate mean values. *Indicates significant difference. NS = not significant; PTB = adult pulmonary TB patients; HA = healthy adult controls; HA+ = HAs with high TBGL-IgG and -IgA titres; HA− = HAs with low TBGL-IgG or -IgA titres or both; TBGL = tuberculous glycolipid; Ig = immunoglobulin; CTB = child TB patients; HC = healthy child controls.

Table 2 Measured parameters and comparison between adult PTB patients and healthy adult controls

Parameter	Adult PTB cases median [range]	Healthy adults median [range]*	*P* value
TBGL-IgG, U/ml	18.7 [0.5–179]	1.5 [0.4–21.4]	<0.001
TBGL-IgA, U/ml	4.9 [0.3–2448]	0.7 [0.08–43.7]	<0.001
Serum IgG, mg/dl	1961 [1433–2835]	1441 [1032–2051]	<0.01
Serum IgA, mg/dl	519 [411–695]	223 [143–861]	<0.01†
KL-6, U/ml	530 [231–1897]	225 [129–592]	<0.001†
Leptin, ng/ml	0.63 [0.13–5.3]	7.7 [0.3–21.6]	<0.001†
sIL-2Rα, ng/ml	2.8 [0.81–15.5]	0.54 [0.1–0.9]	<0.001†
Haemoglobin, gm/dl	12.5 [9.2–14.9]	13.1 [11.1–17.1]	<0.01†
WBC, 10^3/μl	10 [6.8–16.4]	7 [4.6–10.2]	<0.001†
Neutrophil, 10^3/μl	7.08 [5.04–13.78]	3.7 [2.07–6.9]	<0.001†
Lymphocyte, 10^3/μl	1.74 [0.88–3.2]	2.46 [1.85–3.6]	<0.01†
Monocyte, /μl	580 [248–1096]	393 [222–684]	<0.01†
AST, U/ml	25 [15–158]	21 [15–55]	NS
ALT, U/ml	18.5 [7–67]	15.5 [7–75]	NS

*Healthy adults with high titres of both TBGL-IgG and -IgA.
†Significant difference between the two groups ($P < 0.05$).
PTB = pulmonary tuberculosis; TBGL = tubercular-glycolipid; Ig = immunoglobulin; WBC = white blood cells; AST = aspartate aminotransferase; NS = not significant; ALT = alanine aminotransferase.

Table 3 Comparison between TBGL-IgG, TBGL-IgA and combined TBGL-IgG+IgA for their utility in the diagnosis of active pulmonary TB in adults

	TBGL-IgG %	TBGL-IgA %	TBGL-IgG+IgA %	*P* value*
Sensitivity	92	63	63	0.019†
Specificity	54	64	75	0.057

*Statistical difference between TBGL-IgG and TBGL-IgG+IgA groups.
†Significant difference.
TBGL = tuberculous glycolipid; Ig = immunoglobulin.

for the diagnosis of active TB, and the specificities were 54% and 64% (Table 2). Simultaneous detection of both TBGL-IgG and -IgA improved specificity (75%, $P = 0.057$), although sensitivity was significantly lower ($P = 0.019$) than for TBGL-IgG alone (Table 3). To elucidate the cause of high TBGL antibodies in HAs, we therefore further categorised them into two groups: HAs positive for both TBGL-IgG and -IgA (HA+ 7/28, 25%) and others (HA− 21/28, 75%). TBGL-IgG and -IgA titres in the HA+ group were significantly higher than in the HA− group ($P < 0.05$ and $P < 0.01$, respectively) and were not different from those in the PTB groups ($P > 0.05$ for all, Figure 1A). The levels of two antibodies were positively correlated in the HA+ subjects ($r = 0.843$, $P = 0.01$) and among the PTB patients ($r = 0.664$, $P < 0.00005$), but not in the HA− group (Figure 2). TBGL-IgG and -IgA titres were not correlated with those of serum IgG and IgA in the PTB, HA or HA+ groups ($P > 0.05$ for all). No correlation was observed between TBGL-IgG/IgA levels and KL-6 or leptin levels in patients or controls.

In contrast, among the paediatric subjects, only 3/23 (13%) CTB cases and 1/28 (3%) HC had high TBGL-IgG titres, demonstrating the very limited sensitivity (10%) of the assay for the diagnosis of paediatric TB patients. Neither TBGL-IgG nor -IgA titres were significantly different between paediatric cases and controls (Figure 1B).

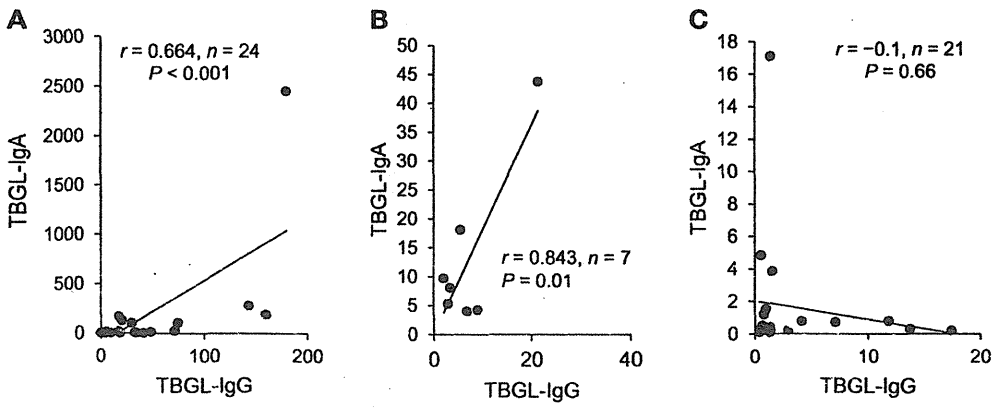

Figure 2 Correlation between TBGL-IgG and -IgA titres. An association was found in **A**) adult PTB patients and **B**) HA+ subjects (healthy adults with high TBGL-IgG and -IgA titres), but not in **C**) HA− subjects (healthy adults with low TBGL-IgG or -IgA titres or both). TBGL = tuberculous glycolipid; Ig = immunoglobulin.

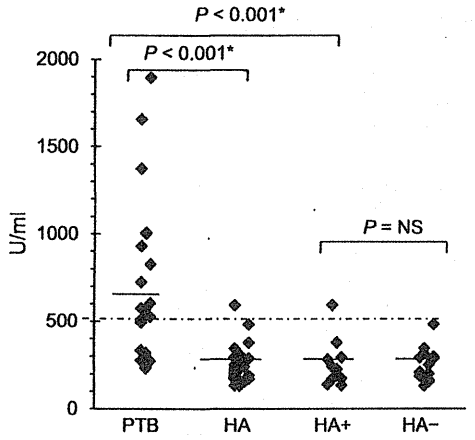

Figure 3 KL-6 titres in adult participants. Dashed line indicates the cut-off value of ⩾500 U/ml. Solid bars indicate mean values. *Indicates significant difference ($P < 0.05$). NS = non-significant; PTB = adult PTB patients; HA = healthy adult controls; HA+ = HAs with high TBGL-IgG and -IgA titres; HA− = HAs with low TBGL-IgG or -IgA titres or both; TBGL = tuberculous glycolipid; Ig = immunoglobulin.

KL-6, leptin and sIL-2Rα serum levels, and various laboratory markers

Serum KL-6 levels were significantly higher in PTB cases than in HAs ($P < 0.01$; Figure 3, Table 2) and were elevated (>500 U/ml) in 14/24 (58%) PTB patients. In contrast, significantly lower leptin titres were found in PTB patients than in HA subjects ($P < 0.001$). Serum IgG, IgA, sIL-2α levels and white blood corpuscle and monocyte counts were significantly higher, whereas the lymphocyte count was significantly lower in PTB cases than in HAs (Table 2). There were no significant differences in measured serum IgG, IgA, KL-6, leptin or other parameters between the HA+ and HA− groups (Table 4).

DISCUSSION

We evaluated TBGL-IgG and -IgA levels in paediatric and adult TB patients and healthy controls in Thailand, a TB-endemic country (TB incidence rate 142/100 000 population).[1]

Poor TBGL-IgG and -IgA reactivity was observed in the paediatric TB patients, consistent with previous findings of low antibody responses among child TB suspects against protein antigens, including purified protein derivative (PPD), 38kDa and HSP60.[18] Low TBGL-Ab titres cannot be explained by low serum IgG or IgA, as these were significantly higher in the CTB than in the HC group (data not shown). Although *M. tuberculosis* infection was not confirmed in most of the CTB cases, their clinical and radiological findings were strongly suggestive of active TB, and all responded well to anti-tuberculosis treatment. The cause of the low antibody responses in children is not clear. However, the underdeveloped immune system in young children might play a vital role against the development of specific adaptive immune responses against TB.

In contrast, TBGL-IgG detection in adult PTB patients was revealed to be highly sensitive (92%), in line with a previous report from Japan.[8] However, increased proportions of positive TBGL-IgG in HAs were accountable for the low specificity (54%), and therefore diminished its usefulness as an active TB diagnostic marker in Thailand. The diagnostic ability of TBGL-IgA was also inadequate, showing lower sensitivity and specificity in the current study. However, the specificity was higher than that of TBGL-IgG. Julean et al. also demonstrated high IgA specificity against four trehalose-containing mycobacterial lipid antigens, including cord factor, in a clinical study.[19]

Table 4 Comparison of clinical and laboratory markers between HA+ and HA−

Parameter	HA+ ($n = 7$) median [range]	HA− ($n = 21$) median [range]	*P* value
Male:female*	5:2	14:7	—
Age, years	38 [23–49]	33 [21–51]	—
TBGL-IgG, U/ml	5.5 [2.1–21.4]	1.3 [0.4–17.4]	<0.05†
TBGL-IgA, U/ml	8 [3.9–43.7]	0.3 [0.08–17.1]	<0.001†
Serum IgG, mg/dl	1367 [1281–1943]	1465 [1032–2051]	—
Serum IgA, mg/dl	192 [166–370]	238 [143–861]	—
KL-6, U/ml	227 [132–592]	223 [129–480]	—
Leptin, ng/ml	8.7 [1.14–19.9]	7.5 [0.3–21.6]	—
sIL-2Rα, ng/ml	0.53 [0.1–0.77]	0.55 [0.1–0.9]	—
Haemoglobin, g/dl	13.1 [12.2–5]	13.7 [11.1–17.1]	—
WBC, 10^3/μl	6.4 [5.5–8.1]	7.3 [4.6–10.2]	—
Neutrophil, 10^3/μl	3.46 [2.3–4.5]	4.1 [2–6.9]	—
Lymphocyte, 10^3/μl	2.5 [2–3.1]	2.4 [1.8–3.6]	—
Monocyte, /μl	402 [384–486]	360 [222–684]	—
AST, U/ml	17 [15–23]	21 [15–55]	—
ALT, U/ml	14 [9–24]	16 [7–75]	—

*Frequency.
†Significant difference between the two groups ($P < 0.05$).
HA+ = healthy adults with high titres of both TBGL-IgG and -IgA; HA− = healthy adults with low titres of either TBGL-IgG or -IgA or both; TBGL = tuberculous glycolipid; Ig = immunoglobulin; WBC = white blood cells; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Elevated TBGL-IgA titres may therefore reflect infection more specifically.

Elevated TBGL-IgG levels were also found in healthy older (>40 years, 17%) and younger adults (<40 years, 5%) in Japan (a non-endemic country); the possibilities of latent TB infection (LTBI) in the TBGL-IgG positive group have already been described by Maekura et al.[8] In this study in Thailand, positive proportions of TBGL-IgG were higher in healthy adults (46%, mean age 34 years) and that of TBGL-IgA was also high (36%). As TBGL-IgG and -IgA titres were not associated with those of serum IgG and IgA, high TBGL antibody titres in endemic HAs cannot be explained by non-specific hyperglobulinaemia. Moreover, none of the HAs had a history of TB. Cross-antibody reactions to other respiratory infections can be excluded, as the HAs were free from respiratory symptoms and had normal CXR findings at the time of enrolment, and bacille Calmette-Guérin vaccination status does not influence antibody production against TDM in adults.[20] It was considered that non-tuberculous mycobacteria (NTM) infection may be responsible for the elevated TBGL-Ab titres in HAs. However, TBGL-IgG titres were reported to increase only in active NTM diseases.[8] Although leptin titres were low in some HAs, none of the TB-related markers, including leptin, KL6 and sIL-2Rα, were different between the HA+ and HA− groups, indicating absence of active disease in HA+. Significant elevations of sensitive TBGL-IgG ($P < 0.05$) and specific TBGL-IgA titres ($P < 0.01$) in HA+ compared to HA− subjects, and the correlation between TBGL-IgG and -IgA titres only in the former group, might be suggestive of the enhancement of TB-specific antibody responses in that group. Although we could not confirm LTBI in HA+ individuals by PPD or an interferon gamma (IFN-γ) release assay (IGRA), a significant association between the QuantiFERON®-TB Gold assay (one of the IGRAs) and the TBGL-IgG assay in healthy adults was documented in our very recent study in the Philippines.[21]

Of note, an increased risk of progression to active TB was correlated with high antibody reactivity to some TB antigens in HIV patients[22,23] and with elevated IFN-γ production to early secreted antigenic target-6 in those with household TB contacts,[24] as the adaptive immune system can recognise antigens produced by early *M. tuberculosis* replication that are thought to be initiated months before the development of active TB.[22,23] However, no follow-up study was undertaken in our HA+ subjects to elucidate risk of active TB.

Taken together, we found that reduced specificity of TBGL-Ab in adult TB patients is due to enhanced humoral immune responses against TBGL in HAs, and that the high TBGL-IgG+IgA reactivity in HA+ controls might be specific and indicative of LTBI. Further extensive evaluation of control subjects from different population groups, including healthy subjects and patients with other pulmonary diseases, and careful follow-up studies, may clarify whether HA+ subjects are at greater risk of development of active TB than in HA− subjects. This might be helpful for the identification of potential markers for early TB diagnosis and the prevention of progressive disease.

Acknowledgements

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III. 結核　免疫抑制患者の結核発症と予防

HIV/エイズ患者

藤田　明

Tuberculosis in HIV/AIDS patients

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Abstract

The recent literatures and guidelines of TB/HIV were reviewed. The Japanese research survey revealed that 0.37 % of active TB patients were estimated to be HIV-positive. Over 60 % of the HIV-infected patients with TB disease were diagnosed as having TB and being HIV-positive almost simultaneously. All HIV-infected patients with diagnosed active TB should be started on TB treatment immediately and active TB patients but not yet on antiretroviral therapy (ART) should be treated with ART within 2 to 8 weeks of starting TB treatment depending on CD4 status. All HIV-infected persons are recommended to receive the test for LTBI and also active TB disease could be prevented by application of WHO three 'I's.

Key words: HIV, tuberculosis (TB), latent tuberculosis infection (LTBI)

はじめに

HIVが米国で初めて報告されてから約30年が経過した今，全世界で2009年の新規HIV感染者が260万人，そして合計では3,330万人の感染者がいる(UNAIDSレポート)．新規HIV感染者数は世界全体では2001年から25％以上減少しているにもかかわらず，日本においては，2010年(速報値)の新規HIV感染者は1,050件で過去3位，新規エイズ患者は453件で過去1位と，流行に歯止めがかかっていない状況である．凝固因子製剤による感染者を加えると累積で2万人に達する数となった．そして，HIV感染者では結核発病の相対リスクは20-37倍といわれる[1]．

1. 世界と日本の疫学

HIV感染症は結核発病の最大の危険因子であり，特にサハラ以南のアフリカ諸国ではHIVと結核の重感染は社会問題にまで発展している．世界的には2008年の結核患者のうち15％の約140万人がHIV陽性であり，HIV/エイズによる死亡例の23％は結核死とされている(2007年)．

一方，日本では結核患者のうちの正確なHIV感染者数を把握するシステムは構築されていないが，2008年の結核入院患者調査研究においてHIV陽性率は0.37％と報告されている[2]．また，HIV感染症の側面からみると，エイズ動向委員会報告におけるエイズ指標疾患件数のうち活動性結核は第5位で，6.5％，外国人におい

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ては第3位，14.8％を占めている(1985-2009年累計)．安岡らによる医療機関対象アンケート調査では，2008年までの活動性結核の累計数はエイズ指標疾患中第4位，9.2％を占めている[3]．

2. HIV合併結核の臨床

全国HIV感染合併結核症アンケート調査報告(2003-06年経験例)[2]と，都立2病院における検討(1997-2007年経験例)[4]によると，結核診断時にHIVが同時期に診断される'いきなりエイズ'例が約6割を占めていた．外国人の比率は比較的多いものの，上記報告例の7割以上は日本人であったことにも留意したい．

HIV合併結核の発病形式には，過去に結核菌に感染したものがHIV感染による免疫機能低下により発病する場合と，小児結核のように初感染からそのまま結核発病に至る場合がある．更に，免疫再構築症候群(immune reconstitution inflammatory syndrome: IRIS)といって，抗HIV薬を開始した後に免疫機能が回復しつつある状態で結核を発症あるいは悪化するものがある．結核治療は継続し，非ステロイド系消炎剤やステロイド薬を投与して対処する．

HIV合併結核はエイズ日和見感染症の代表であるニューモシスチス肺炎と比較して，CD4陽性リンパ球(CD4)数が減少していなくとも発病する点に注意が必要である．CD4数によって結核の臨床像は異なる．CD4数が200(count/mm^3)以下に減少している患者では，初感染結核に類似した臨床像を呈し，また，リンパ節結核(図1)・粟粒結核など肺外結核も起こしやすく，空洞を呈しにくい．結核としての経過も早いことがあるので，症状経過が早いからといって鑑別診断上で結核を除外してはならない．画像上陰影の拡がりの割に排菌量が多い患者もいるので院内感染対策上も注意が必要である．一方，CD4数が500以上と比較的保たれている患者では臨床像は非HIV感染者とほぼ同様と考えてよい．

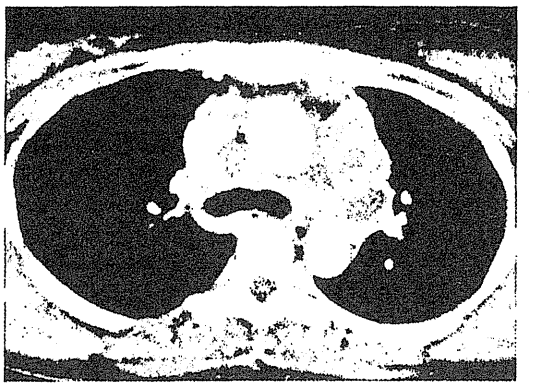

図1 HIV合併結核患者(喀痰抗酸菌塗抹陽性，CD4数50)の胸部造影CT写真
縦隔リンパ節の著明な増大を認め，リング状に造影されている．

3. HIV合併結核の治療

治療の3原則は，①直ちに結核治療を開始する，②抗HIV療法(antiretroviral therapy: ART)を併用し，HIV治療例ではARTを継続し，未治療例では結核治療開始後の適切なタイミングでARTを始める，③IRISを発症しても結核治療とARTを継続する(ステロイド併用を推奨)ことである．

結核治療についてはHREZによる標準治療に準ずる．全治療期間は6-9カ月間とされるが，肺外結核・粟粒結核では治療期間は9カ月間(以上)必要で，最近のメタ解析によると6カ月間では8カ月以上の治療例よりも再発が多い傾向であることが指摘されている[5]．

HIV未治療患者において結核治療開始後にARTをどのタイミングで開始するかについては，抗結核薬および抗HIV薬の副作用，薬物相互作用，IRIS発症の可能性，患者管理の観点から，議論が繰り返されてきた．ガイドラインの発行時期や国・地域によってART開始時期に関する記載が異なっている．南アフリカにおける喀痰塗抹陽性のHIV感染結核患者(CD4数500未満)を対象とした無作為比較試験によると，結核治療開始後2カ月以内あるいは2カ月後の可及的早期にARTを開始した群と，結核治療終了後にARTを開始した群に分けて予後

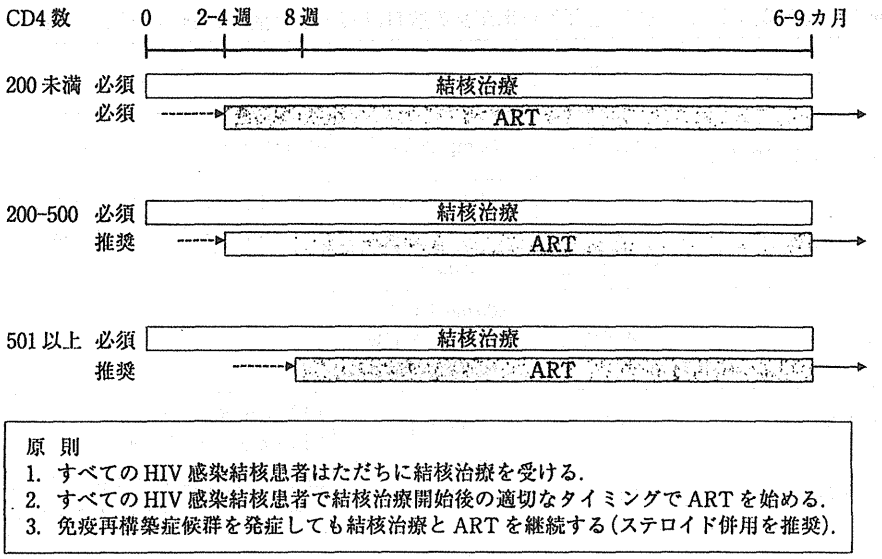

図2 HIV未治療の結核患者における治療原則と抗HIV薬開始の時期(文献7)より改変)
---▸この時期の開始も可, ⟶ 以降継続.

を比較すると，後者の方が結核治療開始後2年までの予後は悪かった(p=0.003)が，副作用については差がなかった[6]．近年はHIV感染症の治療という意味において早期のART開始が推奨されており，米国DHHSの2011年1月のガイドライン[7]ではCD4数に応じてその時期(200未満では結核治療開始後2-4週までに，200-500では遅くとも8週までに，501以上では8週までに)が提示されている(図2)．日本のHIV感染症'治療の手引き'第14版(2010年12月発行)では，'HIVと結核に対する治療の同時開始は勧められない．抗結核療法開始後の抗HIV療法の開始期間については議論が多い'と記載されている．

リファンピシン(RFP)はキードラッグであるが，抗HIV薬と相互作用が多いので注意が必要である．RFPはCYP3A4とCYP2C8/9の酵素誘導作用が強く，多くのプロテアーゼ阻害薬(PI)のAUCを約75％以上低下させる．リファブチン(RBT)は同じリファマイシン系薬剤でありながら酵素誘導がかなり弱いので，RFPが使用できない場合はRBTに置き換える(表1)．RBT特有の副作用としてぶどう膜炎が報告されており，HIV感染結核では，エタンブトールによる視神経障害やサイトメガロウイルス網膜炎などとの鑑別が必要となる．

しかしながら，抗HIV薬のロピナビル/リトナビルとRBTを推奨用量にて投与したがRBTの血中濃度が上がらない例もあるという[8]．また，RFPと併用可能な抗HIV薬エファビレンツ投与においてCYP2B6のジェノタイプによってはエファビレンツの代謝が低下する結果も報告されている[9]．したがって，結核治療中に感受性菌であるにもかかわらず菌陰性化が遅れる場合や抗HIV薬の副作用出現やウイルス学的効果不十分の場合には，薬剤の血中濃度測定を依頼するのも一つの方法である．

4. HIV感染者に対する結核発病の予防

WHOは3つの'I's戦略すなわち，intensified case finding(患者発見)，infection control(感染管理)，isoniazid preventive therapy(INH化学予防)を提唱しているが，HIV感染者に対してはこの戦略を積極的に適用することが勧められる．

潜在性結核感染症(LTBI)の診断にinterferon-γ release assays(IGRAs)が普及しつつある．米国のHIV感染者の日和見感染予防治療ガイドラインでは，①ツベルクリン反応またはIGRAs

表1 抗結核薬(RFP, RBT)と相互作用がある抗HIV薬と併用禁忌の例(文献[7]より改変)

抗HIV薬	RFP	RBT	備 考
プロテアーゼ阻害剤 (リトナビル併用を推奨)	×併用禁忌	150 mg隔日投与	RBTの血中濃度モニターを推奨
エファビレンツ	投与可*	450-600 mg 1日1回	*体重60 kg以上ではエファビレンツ800 mg 1日1回, の意見あり
ネビラピン	×併用禁忌	併用注意	
エトラビリン	×併用禁忌	300 mg 1日1回 プロテアーゼ阻害剤 併用時は禁忌	
ラルテグラビル	併用注意	投与可	RFP使用時にはラルテグラビル1回800 mgを1日2回
マラビロク	×推奨しない	投与可	RBTとCYP3A強阻害剤併用時にはマラビロク1回150 mgを1日2回

検査を行う, ②ハイリスク者, すなわち集団生活者など活動性結核患者との接触危険度が高い場合, 社会的地域的リスクが高い場合には, 毎年検査する, と記載されており, LTBI治療レジメは9カ月間のINH投与でビドキサール併用も推奨されている.

しかし, HIV感染者においては免疫反応性の低下やリンパ球数減少のために, 偽陰性や判定不能となる可能性がある. 著者らの検討[10]ではCD4数が50以上の例においてクォンティフェロン(QFT)は結核感染診断として利用可能であることが示された. しかし, CD4が50未満例においては今後に課題が残されている.

結核接触者健診においてHIV感染者は'ハイリスク接触者'として定義されており, 健診の優先度は高い. 都市部などHIV感染者数が多い地域では健診の機会にもHIV検査を勧めたい. 一方, HIV感染者自身は結核接触者健診の際にHIV陽性を申告しないことも想定されるので, HIV感染者にかかわる医療関係者や機関は, 周囲に結核患者が発生した場合にはハイリスク者として健診を受けて必要な措置を受けることを, HIV感染者には情報提供しておくよう望まれる. 米国ガイドラインでは, HIV感染者で結核患者に接触した場合, QFTなどが陰性であっても結核発病がないことを確認後, LTBIの治療を行うことを推奨している[11].

結核感染管理の詳細は他の稿に譲るが, HIV感染者が通院・入院する医療機関においては結核感染予防対策に配慮がより求められる. そして, HIV陽性率が高い男性同性愛者の集まる店舗への結核予防啓発, 結核ハイリスクであるホームレスや留置場・刑務所などでもHIV感染者が存在することを前提とした対策が必要となりつつある. HIV感染症以外にも糖尿病, アルコール多飲, 胃切除後などの結核発病危険因子を有する患者は'スーパーハイリスク者'として対応すべきであろう.

おわりに

HIV未治療結核合併患者におけるART開始時期については, 臨床試験の新たな結果が公表されると更新される可能性がある. HIV感染症治療の分野は年々進歩しているので, HIV合併結核患者の診療の際には最新のガイドラインを参照されたい.

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2. 結核の現状と問題点—エイズ学会から

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はじめに

演者は日本エイズ学会から本シンポジウム演者の推薦を受け，エイズ診療領域から結核の現状と問題点について報告した。口演内容は過去の学会発表や勧告等をもとに構成したものであり，ここでは概要としてまとめさせ

ていただく。

新規HIV感染者数は世界全体では2001年から09年で25%以上減少しているにもかかわらず，日本においては，2010年の新規HIV感染者は1,075件で過去3位，新規エイズ患者は469件で過去最多と，世界と逆行した傾向が続いている[1]。

潜在性結核感染症から結核を発病する相対リスクは未治療の進行HIV感染症患者では9.5～9.9といわれている[2]。一方，活動性結核は日本におけるエイズ指標疾患の中で4番目に多い。日本において，結核全体の中ではHIV感染症合併例は数としてはまだ目立たないが，重要な課題となりつつある。

日本エイズ学会学術集会における最近の発表の紹介

国内では最近，HIV感染合併結核症例に関してまとまった調査報告が3本報告されている。2009年の第23回日本エイズ学会学術集会では村上らによる全国HIV感染合併結核症アンケート調査報告（2003～2006年に診療された患者，33施設から105例）[3]と，村松らによる都立2病院における検討（1997～2007年に結核を発症した71例）[4]，さらに2010年の第24回日本エイズ学会学術集会では国立国際医療研究センターの千葉らによる検討（1996～2010年に経験された129例）[5]がある。一部に重複症例があると思われる。

発表内容についての詳細はここでは記載しないが，結核診断時にHIVが同時期に診断される「いきなりエイズ」が60～67%と，依然として多く占めていることが示された。従って，結核患者を診察する場合にはHIV感染リスクなども踏まえて積極的にHIV検査を実施すべきである。喀痰塗抹陽性は40～55%で，多剤耐性結核は全国アンケート調査では4例報告されている。外国人の比率は比較的多いものの，上記報告例の71～83%は日本人であったことにも留意したい。抗HIV療法（antiretroviral therapy: ART）開始後に免疫再構築症候群を発症する頻度は7～8%であった。

治療ガイドラインと臨床試験結果について

HIV結核診療において最大の課題は，HIV未治療患者において結核治療開始後にARTをいつのタイミングで開始するか，である。近年はHIV感染症の治療という意味においては，早期のART開始が推奨されている。しかし，結核合併例においては，薬剤の副作用，薬物相互作用，免疫再構築症候群の可能性の観点から，ART開始時期について議論が続いている。

米国DHHSの2011年1月のガイドラインにおいて，CD4陽性リンパ球数（CD4数）200未満では結核治療開始2～4週以内にART開始が必要で，200～500では遅くとも8週後までに推奨するとしている[6]。

南アフリカにおける喀痰塗抹陽性のHIV感染結核患者（CD4数500未満）を対象としたART開始時期に関する無作為比較試験（SAPIT試験）によると，結核治療開始後2カ月以内あるいは2カ月後の可及的早期にART開始した群と，結核治療終了後にART開始した群に分けて予後を比較すると，後者のほうが結核治療開始後2年までの予後は悪かった（p=0.003）が，副作用については差がなかった[7]。2011年の「レトロウイルスと日和見感染症会議（CROI）」で報告されたところによると，SAPIT試験の早期ART群を解析したところCD4数50未満の例では2カ月以内にARTを開始したほうがエイズ発症または死亡リスクが低い傾向にあった。

今後もガイドラインは更新されると思われるが，実地臨床においてはまず結核治療を開始してから，CD4，抗結核薬の副作用，患者の状態，などの経過をみたうえで，遅滞なくARTを開始することが妥当であろう。

結核発病の予防とThree I's

HIV感染者からの新たな結核発症を防ぐ手段の一つは，潜在性結核感染症（LTBI）の早期発見・治療である。CDCやWHOはHIV感染者に対しての結核スクリーニングを推奨している。WHOによるThree I's（患者発見強化，INHによる予防治療，結核感染コントロール）を適用することが重要である。

発病予防の観点について，演者は2009年の第84回日本結核病学会総会ミニシンポジウム「ハイリスク者の結核発病予防　4．HIV感染症からの結核発病予防について」において報告している[8]。また，臨床の現場においてはインターフェロンγ遊離試験（IGRAs）が普及しつつあるが，HIV感染者における反応性低下の問題，最近の第3世代による検討結果については，第86回日本結核病学会総会ミニシンポジウム「IGRAの新しい展開」において述べたので本稿では割愛する。

おわりに

HIV感染合併結核への対応については上述の内容以外にも，免疫再構築症候群を含む治療上の課題，医療機関における診断と院内感染対策，多剤耐性結核への警戒，などの重要な課題がある。また，ガイドラインは毎年更新されるので，最新のガイドラインを参照してほしい。

まとめ

HIV感染は結核発病リスクを著しく高める。最近の国内報告においても，結核診断時にHIVが同時期に診断される例が依然として多い現状であった。結核治療開始後

のART開始時期についてはCD4数に応じて推奨されているが，新たな海外臨床試験の結果によりガイドラインはアップデートされるだろう。

謝　辞

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3. 透析患者における結核の現状と問題点

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透析患者では，感染症が20.8%と死亡原因の第2位で，近年横ばいである心不全に比べて増加傾向であり，新たに透析を開始した患者の死亡原因としては，感染症は26.4%で第1位である[1]。その要因としては，透析患者にともなう免疫能低下，高齢化，糖尿病が原因疾患として多いことなどがあげられる。

なかでも，結核感染については，透析患者は一般人と比べて，報告により差があるが，約2倍～25倍感染のリスクが高いとされる[2)~5)]。透析患者では，細胞性免疫能が低下しており，初感染より長期間経過後，結核菌が再び活動し発病する内因性感染による肺外結核が全結核の約半数を占めるほど多いこと，免疫能が低下しているため血行性伝播による粟粒結核が多いこと，透析導入1年以内の発症が多いこと，高齢者が多いことなどが特徴である[5)]。

また，透析患者の結核診断は，培養検査で陽性にでることが少ないことや，免疫能の低下によりツベルクリン反応の陽性率が低いことなどによりしばしば困難である[6)]。最近，BCGの影響を受けないQuantiFERON TB-2G（QFT）の結核診断における有用性が注目されている[7)]。これは免疫反応を利用した結核の補助診断であるが，免疫能が低下した透析患者でも有用性が報告されている。Inoueら[8)]は，透析患者162例にQFTを施行し，感度100%，特異度89.7%と良好な成績を報告している（Table 1）。

透析施設での結核蔓延の報告は少ないが，2003年アメリカ，ネバダ州で，技士が3カ月間肺結核で，透析スタッフの19%，透析患者の15%でツベルクリン試験が陽性であったと報告され，閉鎖した空間で長期間免疫能の低下した患者が大勢いる透析施設は，結核蔓延のリスクが高いことが示されている[9)]。

また，透析患者の結核の予後について，佐々木ら[5)]は，肺結核32例中結核死が9.4%，他の原因の死亡が9.4%で，結核菌陽性患者の死亡が30%と高く，肺外結核36例では，結核死が5.6%，他の原因による死亡が13.9%で，特に結核性胸膜炎の結核死が16.7%と高いことを報告している。

Table 1　Results of QFT in hemodialysis patients

	Active TB	Non-TB	Previous TB	Total
Positive QFT	8	10	10	28
Negative QFT	0	87	8	95
Indeterminate	0	34	5	39
Total	8	131	23	162

(sensitivity 100% ; specificity 89.7%)
Inoue T, et al: Nephrol Dial Transpl, 2009.