② LTBI 患者

　　◎全ての LTBI 患者の管理検診はいらないのではないか。

- 発病さえしていない LTBI を治療して発病率を低下させたのに，さらに治療後の健診を行うというのは理解し難い。
- 接触者健診で発病者の多い場合は管理検診をする。
- 管理健診を行う対象者を選別してもよいのではないか。
- エビデンスがないので，例えば保健所にアンケート調査をして検討するのはどうか。
- LTBI の治療した人の登録期間が治療しない人の登録よりも長いというのはおかしい。
- 菌のタイプ（VNTR などで）によって管理検診の方法を変える。
- 年齢層によって管理検診の方法を変える。
- NNS（Number Needed to Screen）の観点からも LTBI 治療後健診は受け入れ難い。
- 保健所長の判断で管理検診をするかどうかを決める。
  - ➢　⇒そのためには学会からの提言（例：手引きを作成する）のような形で判断をする方法についてバックアップする必要がある。
- 国際的には発病治療後の一律な健診は不要とされる中，LTBI の管理健診を推奨しているガイドラインもない。
- むしろ 2013 年に CDC が公表した LTBI に関する一般診療科向けガイドには，治療後のフォローアップは「not indicated（適応なし）」と明記されている。
- 保健所に聞いてみると LTBI 患者の管理健診はかなり負担となっている。

参加者
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Ⅲ　研究成果の刊行に関する一覧表

別紙4

研究成果の刊行に関する一覧表

雑誌

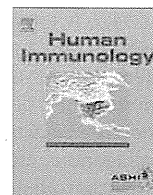| 発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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Ⅳ　研究成果の刊行物・別刷(一部)

ELSEVIER

Age-dependent association of mannose-binding lectin polymorphisms with the development of pulmonary tuberculosis in Viet Nam

CrossMark

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ABSTRACT

Mannose-binding lectin (MBL) binds to pathogens and induces complement-mediated opsonophagocytosis. Although the association between *MBL2* polymorphisms and tuberculosis (TB) has been studied in various populations, the results are controversial. We explored the stages of TB associated with *MBL2* polymorphisms. *X/Y* (rs7096206) and *A/B* (rs1800450) were genotyped in 765 new patients with active pulmonary TB without HIV infection and 556 controls in Hanoi, Viet Nam. The *MBL2* nucleotide sequences were further analyzed, and plasma MBL levels were measured in 109 apparently healthy healthcare workers and 65 patients with TB. Latent TB infection (LTBI) was detected by interferon-gamma release assay (IGRA). The *YA/YA* diplotype, which exhibited high plasma MBL levels, was associated with protection against active TB in younger patients (mean age = 32) $\leq$ 45 years old (odds ratio, 0.61; 95% confidence interval, 0.46–0.80). The resistant diplotype was less frequently found in the younger patients at diagnosis ($P = 0.0021$). *MBL2* diplotype frequencies and plasma MBL levels were not significantly different between the IGRA-positive and -negative groups. *MBL2 YA/YA* exhibited a protective role against the development of TB in younger patients, whereas the *MBL2* genotype and MBL levels were not associated with LTBI. High MBL levels may protect against the early development of pulmonary TB after infection.
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1. Introduction

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB) in humans and presumably infects a third of the world's population. *Mtb* establishes a persistent infection in immune cells such as macrophages, and 5–10% of immunocompetent individuals develop active TB during their lifetime, whereas the others limit infection by successful containment of *Mtb* in granulomas. The innate immune response induces activation of the T

helper 1 (Th1)-type immune system and plays an important role in host defense against the development of TB [1]. Many studies have reported the association between TB and polymorphisms of host genes related to innate immunity [2].

Mannose-binding lectin (MBL) is an acute-phase serum protein in the collectin family that recognizes a pathogen by its carbohydrate-recognition domains [3]. MBL is synthesized in the liver and circulates in the form of oligomers complexed with MBL-associated serine proteases (MASPs). Upon binding to the sugar moieties on the pathogen surface, MASPs are activated to initiate the lectin pathway of complement activation, which results in opsonization and phagocytosis or lysis of microorganisms. Besides its direct action as an opsonin and its key role in the lectin pathway, MBL may modulate inflammatory responses and immune activation [4].

MBL is encoded by *MBL2*, which is located on chromosome 10, and six *MBL2* single nucleotide polymorphisms (SNPs) are associated with serum levels and/or functions of MBL. Three nonsynonymous nucleotide substitutions in exon 1 change the wild *A* allele to the three variant alleles (*A/B*, *A/C*, and *A/D*), which disrupt the collagenous structure and the formation of functional oligomers. The other alleles, *H/L*, *X/Y*, and *P/Q*, are distinguished by the SNPs in the promoter and 5′-untranslated regions, and the *X* allele shows the lowest transcriptional activity among them [5]. Because of strong linkage disequilibrium, seven haplotypes are commonly observed and often classified into three groups of higher producing (*HYPA*, *LYPA*, and *LYQA*), lower producing (*LXPA*), and nonfunctional (*LYPB*, *LYQC*, and *HYPD*) haplotypes.

These genetic variations that result in MBL deficiency are associated with a wide variety of diseases, including respiratory tract infections, presumably because of the leak of circulating MBL into inflamed airways [6]. However, *MBL2* polymorphisms show conflicting results and confer either resistance or susceptibility toward pulmonary TB [2]. According to some studies, MBL deficiency is associated with protection against TB disease, raising the hypothesis that the uptake of microorganisms by phagocytes is enhanced by MBL binding, which results in the promotion of infection by intracellular pathogens [7,8]. In contrast, other investigators have suggested that high MBL levels have a protective effect against TB [9,10].

Immune responses control *Mtb* infection in the latent phase, but *Mtb* is reactivated from an immunological equilibrium to develop TB disease [11]. The persistence of the latency period in adult patients with TB varies greatly among individuals, and this may reflect the duration of successful *Mtb* containment. During this process, it is believed that pathogenic factors, including different genotype strains, may also play a role [12]. We found a protective role of the interferon gamma receptor 2 gene (*IFNGR2*) polymorphism against TB; furthermore, we found that the resistant alleles tended to be less frequent in younger patients at diagnosis when we investigated polymorphisms in the Th1-immune response genes in Vietnamese patients with TB [13]. Grant et al. also found an age-dependent association of thymocyte selection-associated high mobility group box gene (*TOX*) variants in Morocco and Madagascar and highlighted the importance of age at TB diagnosis, which is correlated with the duration of the latency period in endemic areas [14]. The inconsistent association between *MBL2* and TB in different studies may be attributable to the different stages of *Mtb* infection from latent TB infection (LTBI) to TB disease; therefore, in the present study, we explored whether *MBL2* polymorphisms or MBL levels are associated with the development of active TB in apparently immunocompetent patients of various ages or the stage of LTBI in Viet Nam, a country with high TB prevalence.

2. Materials and methods

2.1. Study population

The patients and controls were recruited from Hanoi, Viet Nam [13,15,16]. In total, 832 patients (age, 41 ± 14.4 years; 77.6% males) without a previous TB episode were recruited immediately after the diagnosis of new smear-positive pulmonary TB was made. Pulmonary physicians treated them with anti-TB drugs according to the guidelines of the national TB program. Fifty-three HIV-positive patients with TB, four with no information about HIV status, and nine with missing age data were excluded from further analysis. *Mtb* genotyping method was described elsewhere [16]. Beijing genotype of *Mtb* isolates was distinguished from non-Beijing genotype in 429 TB patients with no HIV infection.

The control group for this genetic association study consisted of 556 healthy volunteers (age, 36 ± 10.3 years; 48.6% males) who

had the same ethnicity and were residents in the same area of Hanoi city. Information of their LTBI status was not available, but 109 disease-free healthcare workers (HCWs; age, 34 ± 10.1 years; 23.9% males) were also recruited and their LTBI status was assessed by an enzyme-linked immunosorbent assay (ELISA)-based interferon gamma release assay (IGRA; QuantiFERON-TB Gold In-Tube™, Cellestis, Victoria, Australia) [17]. All were unrelated Vietnamese. Informed consent was obtained from all participants. The study protocol was approved by the ethics committees of the Ministry of Health, Viet Nam (4481/QD-BYT, 2529/QD-SYT), the National Center for Global Health and Medicine (NCGM-A-000185-00, 63), and the Research Institute of Tuberculosis (RIT/IRB25-1, 25-2), Japan.

2.2. Haplotype analysis of MBL2 SNPs in the Vietnamese HCWs and patients with TB

Genomic DNA samples from the 109 HCWs and 156 patients with TB were randomly subjected to polymerase chain reaction (PCR) amplification of the *MBL2* promoter and exon 1 regions with the primers 5′-GACCTATGGGGCTAGGCTGCTGAG-3′ and 5′-CCCC AGGCAGTTTCCTCTGGAAGG-3′ using TaKaRa LA Taq (TaKaRa, Shiga, Japan). The amplified products (1112 bp) were purified and sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the 3130xl Genetic Analyzer (Applied Biosystems). The synonymous SNP in exon 4 (rs930507) was amplified by PCR with the primers 5′-CTTTG TACCAGTCTGTCTGTTCAC-3′ and 5′-GGCCTGGAACTTGACACACAAG GC-3′ and genotyped using the restriction fragment length polymorphism method with *Ban* II (TaKaRa).

2.3. Plasma MBL level assay

Plasma MBL levels in samples were assayed by ELISA (Human MBL Quantikine ELISA Kit; R & D Systems, Minneapolis, MN, USA), which can specifically detect oligomeric forms of natural human MBL in serum, heparinized plasma, and EDTA plasma samples. Whole blood was divided into an EDTA tube and a negative control tube for the IGRA (nil tube). Plasma was separated immediately from the EDTA tube, whereas the nil tube was incubated at 37 °C for 16–24 h and centrifuged to separate the plasma. MBL levels in the plasma from the two procedures were compared for 31 individuals, and the coefficient of variation was calculated as 13.2%. Because incubation with heparin did not affect the results considerably, MBL levels were assayed in plasma supernatants from the control tubes for 109 HCWs and 65 patients with TB before the initiation of anti-TB treatment (0 month), after the initial phase of treatment (2 months), and at the end of treatment (7 months). These subjects were selected randomly from the abovementioned 156 patients.

2.4. MBL2 X/Y and A/B genotyping

X/Y (rs7096206) and *A/B* (rs1800450) polymorphisms were amplified in one DNA fragment by PCR using primers 5′-ACCTGG GTTTCCACTCATTCTCAT-3′ and 5′-CCCCAGGCAGTTTCCTCTGGAA GG-3′. An amplified product of 623 bp was digested with *Btg* I (New England Biolabs, Ipswich, MA, USA) to genotype *X/Y* or with *Ban* I (New England Biolabs) to genotype *A/B*, and they were electrophoresed on 2% agarose gels with ethidium bromide. Genotypes were determined by the length of the digested PCR products (*Y* allele with 540 bp after *Btg* I digestion and *A* allele with 536 bp after *Ban* I digestion).

2.5. Statistical analysis

Frequencies of haplotypes containing multiple polymorphic sites were estimated by Haploview ver. 4.2 [18]. Each individual's haplotypes were estimated by the PHASE program v.2.1 [19]. The association between the *MBL2* polymorphisms and plasma MBL levels after logarithmic transformation was analyzed using a multiple regression model. Differences in levels among the *MBL2* diplotype-based groups were further assessed by one-way analysis of variance (ANOVA) with the Tukey–Kramer method. Hardy–Weinberg exact tests were conducted to examine whether the genotype frequencies in the populations were compatible with Hardy–Weinberg equilibrium. TB development associated with a particular *MBL2* diplotype was assessed by odds ratios (ORs) and 95% confidence intervals (CIs) using a logistic regression model in which the interaction term between a particular MBL diplotype and age was also considered. The age-dependent trend for the presence of the *MBL2* diplotype in the patient population and their subgroups divided by *Mtb* genotypes was assessed by the change in odds at the 10-year interval in another logistic model.

Plasma MBL levels between groups with and without LTBI, as estimated by the IGRA results, were compared by analysis of covariance after adjusting for age and gender. The overall effects of *MBL2* diplotype, age, and gender on plasma MBL levels throughout the anti-TB treatment period were assessed using a random intercept model. All statistical analyses were performed using JMP ver. 9.0.0 (SAS Institute, Cary, NC, USA). Stata ver. 11 (Stata-Corp, College Station, TX, USA) was also used for the random intercept model analysis. *P*-values of <0.05 were considered statistically significant.

3. Results

3.1. Distribution of MBL2 haplotypes in the Vietnamese population

The *HYPA*, *LYPA*, *LYQA*, *LXPA*, and *LYPB* haplotypes in the Vietnamese HCWs and TB patients were estimated by the PHASE program (Table 1). The *LYQC* and *HYPD* haplotypes were not found. Thus the five haplotypes were observed in the Vietnamese population and they were identical to those directly determined by long-range PCR from other Asian populations [20,21]. Promoter −503 A/G SNP (rs7100749) was also polymorphic in the Vietnamese population; the −503 A allele was strongly associated with the *LYPA* haplotype. No novel mutation was found in the 156 patients with TB, including the randomly selected 65 individuals whose plasma MBL levels were measured afterwards in this study. Exon 4 synonymous C/G SNP (rs930507) is known to affect the MBL level of *LXPA* [22], but the *LXPA-rs930507* G haplotype was rarely found; the estimated frequency was 0.02 in the Vietnamese population. Therefore, rs930507 was excluded from further analysis.

Fig. 1. Mannose-binding lectin *(MBL)2* diplotypes and plasma MBL levels in healthcare workers. Box-and-whisker plots of plasma MBL levels according to *MBL2* diplotypes of *X/Y* and *A/B* polymorphisms in healthcare workers. Each dot represents an individual. Differences in levels among *MBL2* diplotype-based groups were assessed by one-way analysis of variance.

3.2. MBL2 haplotypes associated with plasma MBL levels in the population

In agreement with previous studies [3], *X/Y* and *A/B* polymorphisms were strong determinants of plasma MBL levels in the multiple regression model (*P* < 0.0001 and *P* < 0.0001). *H/L* was only weakly associated with MBL in the same model (*P* = 0.0378). Neither *P/Q* nor age was associated with MBL levels in these 109 Vietnamese HCWs (*P* = 0.1566 and *P* = 0.2484). On the basis of these findings, the *MBL2* diplotypes were divided into three groups according to MBL levels, i.e., high (*YA/YA*), intermediate (*XA/YA*), and low or deficient (*XA/XA*, *YA/YB*, *XA/YB*, and *YB/YB*; Fig. 1). MBL levels were actually different among the three groups (*P* < 0.0001 by ANOVA), and the difference was significant between any two of the three groups (data not shown). The plasma level that defines MBL deficiency has not been clearly determined, but a recent study with a large sample size (*n* = 1037) using the same ELISA kit adopted ⩽1000 ng/ml as the category for partial and severe MBL deficiency [23]. Our classification almost matched with this definition, as MBL levels were >1000 ng/ml in 38/42 (90.5%) of the *YA/YA*-carrying individuals and ⩽1000 ng/ml in 45/45 (100.0%) of the *XA/XA*- or *B*-carrying individuals.

3.3. YA/YA diplotype associated with protection against TB in younger patients

Because plasma MBL levels largely depended on the combinations *X/Y* and *A/B*, frequencies of these variants were compared between all patients with TB and the controls. The genotype

Table 1
Estimated haplotypes and their frequencies in the Vietnamese healthcare workers (*n* = 109) and TB patients (*n* = 156).

Haplotype	Promoter								Exon1		HCWs		TB patients	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	*n*	Frequency	*n*	Frequency
	−550 *H/L*						−221 *X/Y*		+4 *P/Q*	G54D *A/B*				
HYPA	G	G	A	A	A	ins	G	C	C	G	97	0.445	136	0.436
LYPA	C	A	A	A	A	ins	G	C	C	G	16	0.064	24	0.077
LYQA	C	G	C	G	G	del	G	T	T	G	21	0.096	26	0.083
LXPA	C	G	A	A	A	ins	C	C	C	G	46	0.211	74	0.237
LYPB	C	G	A	A	A	ins	G	C	C	A	38	0.174	52	0.167

(1) rs11003125, (2) rs7100749, (3) rs11003124, (4) rs7084554, (5) rs36014597, (6) rs10556764, (7) rs7096206, (8) rs11003123, (9) rs7095891, (10) rs1800451.
Abbreviations: ins, insertion; del, deletion.
Statistically significant difference between the haplotype frequencies of 156 TB patients and those of 109 HCWs was not observed.
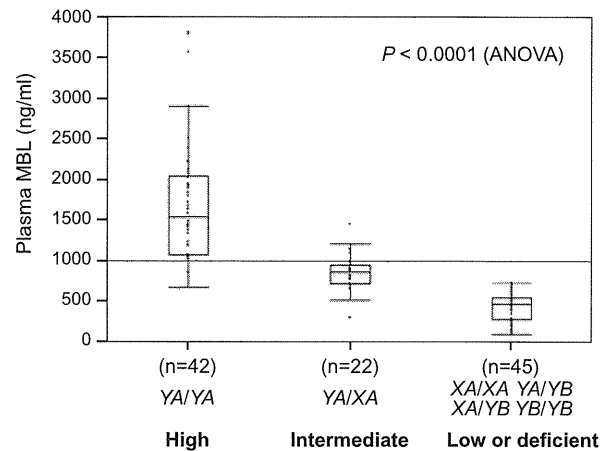
Table 2
MBL2 diplotypes in controls and patients with TB.

MBL levels	Diplotype	Full population			≤45 years			>45 years		
		Controls (n = 556)	TB (n = 765)	OR (95% CI)	Controls (n = 436)	TB (n = 457)	OR (95% CI)	Controls (n = 120)	TB (n = 308)	OR (95% CI)
		No. (%)	No. (%)	[P value]	No. (%)	No. (%)	[P value]	No. (%)	No. (%)	[P value]
High	YA/YA	224 (40.3)	262 (34.2)	**0.77 (0.62–0.97) [P = 0.028]**	178 (40.8)	135 (29.5)	**0.61 (0.46–0.80) [P = 0.0004]**	46 (38.3)	127 (41.2)	1.13 (0.73–1.74) [P = 0.66]
Intermediate	YA/XA	148 (26.6)	231 (30.2)		122 (28.0)	154 (33.7)		26 (21.7)	77 (25.0)	
Low	XA/XA	35 (6.3)	50 (6.5)		23 (5.3)	30 (6.6)		12 (10.0)	20 (6.5)	
	YA/YB	98 (17.6)	142 (18.6)		76 (17.4)	91 (19.9)		22 (18.3)	51 (16.6)	
	XA/YB	33 (5.9)	56 (7.3)		24 (5.5)	34 (7.4)		9 (7.5)	22 (7.1)	
	YB/YB	18 (3.2)	24 (3.1)		13 (3.0)	13 (2.8)		5 (4.2)	11 (3.6)	

TB development associated with YA/YA was assessed by odds ratios (OR) with non-YA/YA as a reference. P values and ORs with confidence intervals (CIs) shown in bold type are statistically significant.

Table 3
Tendency for the presence of high or low MBL level diplotypes in the order of age strata: subgroup analysis by Mtb strains.

Age (years)	TB (n = 765)	(%)	Beijing (n = 250)	(%)	Non-Beijing (n = 179)	(%)
High MBL level (YA/YA) diplotype (n/N)						
Total	262/765	(34.2)	87/250	(34.8)	69/179	(38.5)
16–25	34/122	(27.9)	10/39	(25.6)	9/28	(32.1)
26–35	51/168	(30.4)	19/68	(27.9)	7/32	(21.9)
36–45	50/167	(29.9)	19/53	(35.8)	9/29	(31.0)
46–55	73/180	(40.6)	27/64	(42.2)	26/47	(55.3)
56–65	33/81	(40.7)	7/19	(36.8)	10/24	(41.7)
66-	21/47	(44.7)	5/7	(71.4)	8/19	(42.1)
OR per 10-year change (95% CI)	**0.85 (0.76–0.94)**		**0.77 (0.63–0.94)**		**0.82 (0.67–0.99)**	
Low MBL levels (XA/XA and YB-positive) diplotypes (n/N)						
Total	272/765	(35.6)	85/250	(34.0)	67/179	(37.4)
16–25	50/122	(41.0)	19/39	(48.7)	12/28	(42.9)
26–35	58/168	(34.5)	27/68	(39.7)	9/32	(28.1)
36–45	60/167	(35.9)	16/53	(30.2)	13/29	(44.8)
46–55	59/180	(32.8)	21/64	(32.8)	14/47	(29.8)
56–65	27/81	(33.3)	2/19	(10.5)	12/24	(50.0)
66-	18/47	(38.3)	0/7	(0.0)	7/19	(36.8)
OR per 10-year change (95% CI)	1.05 (0.94–1.16)		**1.43 (1.16–1.78)**		0.98 (0.81–1.19)	

The trend for the presence of the corresponding diplotype was calculated as an odds ratio in a logistic model when the patients were 10 years younger at the time of diagnosis. The corresponding P-values are also shown in the Section 3. The bold font denotes statistically significant odds ratio (OR); CI, confidence interval. The frequencies of diplotypes in TB patients with Beijing genotype and with non-Beijing genotype are shown in Supplementary Table.

distribution of the X/Y and A/B polymorphisms did not deviate from the Hardy–Weinberg equilibrium. We found that YA/YA was significantly associated with protection against TB (P = 0.028, OR, 0.77; 95% CI, 0.62–0.97; Table 2), even after adjustment for gender (data not shown). When YA/YA was further analyzed together with age (≤45 years or >45 years), the genotype–age interaction was significant (P for interaction = 0.018). Therefore, the patients with TB and the corresponding controls were divided into two subgroups. A significant negative association was observed between TB and YA/YA only in the subgroup (mean age = 32) equal to or younger than 45 years old (P = 0.0004, OR, 0.61; 95% CI, 0.46–0.80; Table 2).

Considering the importance of the genotype–age interaction in TB pathogenesis, we investigated the age-dependence of the YA/YA frequencies in the patient group. When the patients were 10 years younger at the time of diagnosis, the trend of carrying YA/YA was significantly lower (P = 0.0021; Table 3), whereas such age-dependency was not observed in the control subjects (data not shown). The frequencies of B-allele-positive or XA/XA diplotypes resulting in low or deficient MBL levels were not significantly different between patients and controls, regardless of age stratification (Table 3).

Genetic information about the pathogen was available for 429 patients with TB among the HIV-negative cases. Beijing genotype Mtb strains are known to infect Asians and are spreading

worldwide [24]. These strains were isolated from 250 patients in our Vietnamese study, and non-Beijing strains were found in 179 patients [16]. Regardless of Mtb genotype, the YA/YA-resistant diplotype was significantly less frequent when patients were 10 years younger at the time of diagnosis (P = 0.02 in the Beijing strain group and P = 0.04 in the non-Beijing strain group; Table 3), whereas low-level or deficient MBL2 diplotypes containing the B-allele or XA/XA were more frequent in the younger age group only among patients with TB of the Beijing genotype strains (P = 0.0014; Table 3). When clinical information from the 506 patients with TB was analyzed, MBL2 variations were not associated with TB severity, as estimated by the area of lung infiltration or recurrence of TB after therapy (data not shown).

3.4. Plasma MBL levels and diplotype frequencies not associated with the status of LTBI

Among the 109 HCWs, 68 (age, 33 ± 9.5 years; 22.1% males) were IGRA-negative while 41 (age, 35 ± 11.1 years, 26.8% males) were IGRA-positive. Plasma MBL levels and MBL2 diplotype frequencies were not significantly different between IGRA-positive and -negative HCWs (Fig. 2), even after adjusting for age and gender (data not shown).
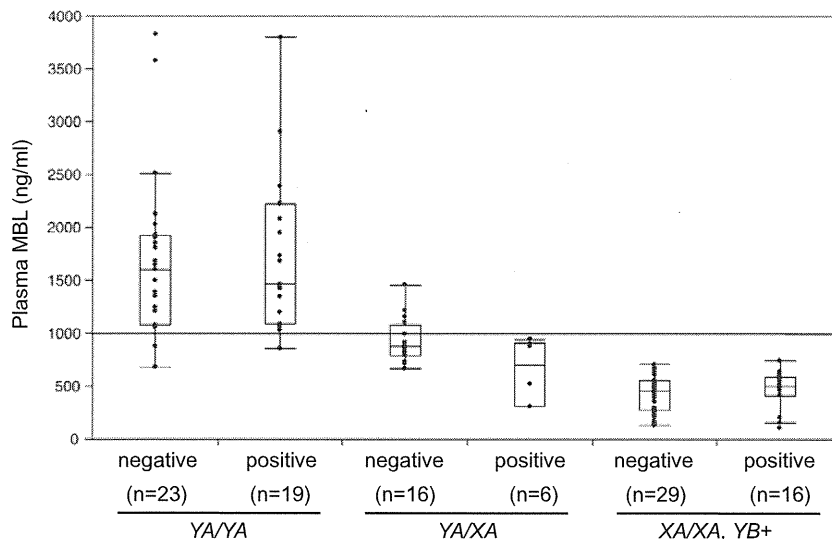
Fig. 2. Plasma mannose-binding lectin (MBL) levels in healthcare workers with and without latent tuberculosis infection (LTBI). Box-and-whisker plots of plasma MBL levels according to interferon gamma release assay (IGRA)-positive and -negative healthcare workers. Each dot represents an individual. Plasma MBL levels between groups were compared after adjusting for age and gender by analysis of covariance.
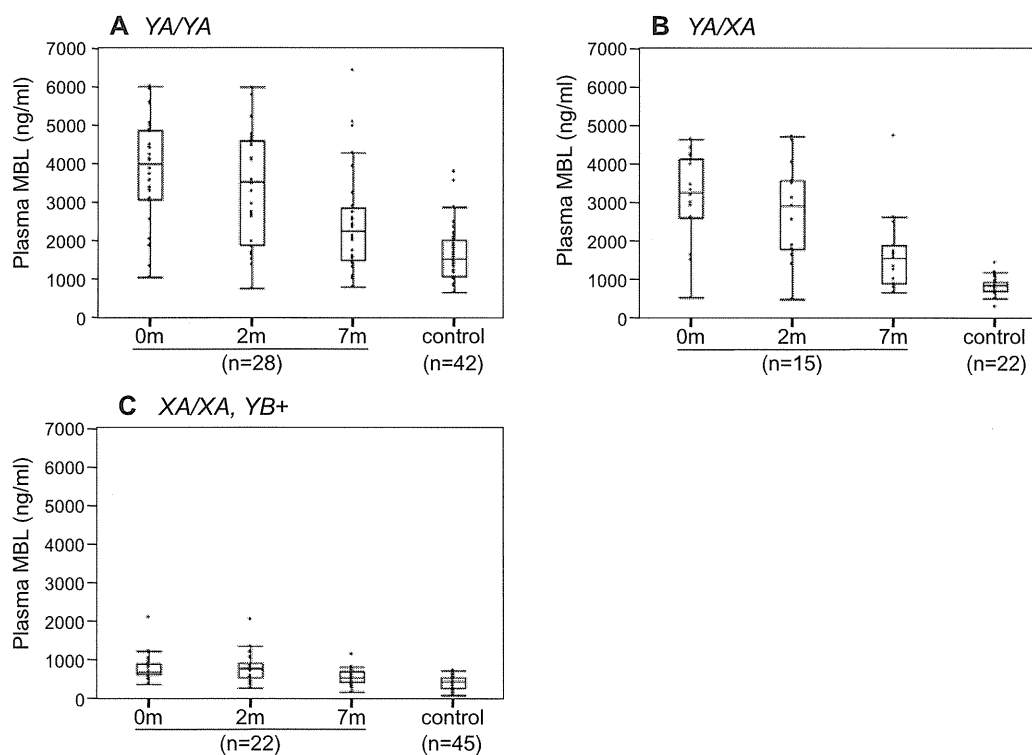

Fig. 3. Time-dependent changes in plasma mannose-binding lectin (MBL) levels in patients with tuberculosis (TB). Box-and-whisker plots of plasma MBL levels from the patients with TB [before (0 month), during (2 months), and at the end of (7 months) treatment] and from healthcare workers (control) with the *YA/YA* diplotype and high MBL levels (A), *YA/XA* diplotype with intermediate MBL levels (B), and *XA/XA* and YB-positive diplotypes with low or absent MBL levels (C). Each dot represents an individual. The overall effects of *MBL2* diplotype, age, and gender on plasma MBL levels throughout the anti-TB treatment period were assessed by using a random intercept model.

3.5. MBL diplotype-dependent plasma MBL levels and their changes during anti-TB treatment

MBL is an acute-phase reactant protein [25,26], and its plasma level is elevated in patients with TB [9]. We assayed MBL levels in serial plasma samples from 65 patients with TB [before (0 month), during (2 months), and at the end of (7 months)

anti-TB treatment]. Plasma MBL levels were the highest before treatment and decreased during treatment (Fig. 3). MBL levels were significantly affected by *MBL2* diplotype throughout the treatment course ($P < 0.0001$), whereas age and gender did not affect the MBL levels ($P = 0.452$ and $P = 0.866$). The MBL concentrations at 7 months were still significantly higher than those of controls in any diplotype groups.

4. Discussion

We found that *YA/YA* associated with high plasma MBL levels was associated with protection against the development of new pulmonary TB. This result is consistent with that of a previous study on European descendants, which reported that *HYA/HYA* subjects are protected against TB [10]. Interestingly, the association was found only in younger patients when TB patients were stratified by age in our study. Nongenetic factors that may directly affect MBL levels include inflammatory condition and age, and MBL levels are higher in children than in adults [3]. In contrast, no association has been found between age and MBL levels among adults in some studies [27,28], whereas others reported that serum and plasma MBL levels in healthy adults decrease with age [29,30], although the levels were not stratified by *MBL2* genotypes in those studies. We did not find a significant age-dependent difference in MBL levels in either adult patients with TB or the control group, whereas we clearly demonstrated *YA/YA* diplotype-dependent effects on MBL levels even in the disease state during anti-TB treatment.

The protective role played by high MBL levels only in the younger patients with TB may be related to the complicated immune balance involved in TB development, which occurs within a shorter duration after *Mtb* infection in younger patients. On the other hand, older patients may have maintained LTBI status and *Mtb* in granulomas for a longer time. Because previous studies have suggested that the interaction between MBL and pathogens enhances proinflammatory cytokine production [31–33], high MBL levels may contribute to the maintenance of *Mtb* containment. Moreover, MBL affects monocyte and dendritic cell function at high levels without forming a complex with pathogens [34–37]. Although high MBL levels may limit *Mtb* infection into LTBI status during the younger days of a patient, the decline in the immune response with aging may result in the development of TB disease. The interaction between MBL and host cells and the subsequent immunological response should be elucidated in both the young and the elderly. The effects of associated SNPs in other genetic studies of TB are also restricted in young patients with TB [13,14,38]. Although the mechanism of age-dependent association may be different among genes, studies on the association between host genetic polymorphisms and TB should carefully consider the effects of age.

It has been speculated that the lack of MBL as an opsonin may suppress *Mtb* uptake by macrophages and prevent infection. However, we did not find significantly different plasma MBL levels and the frequencies of *MBL2* genotypes between HCWs with and without LTBI. Therefore, high MBL levels may not promote the establishment of LTBI, but *YA/YA* may confer protection against the development of TB. This is a new concept because the relationship between the *MBL2* genotype and TB infection, as assessed by IGRA or the tuberculin skin test, has not been clearly investigated so far. A limitation of the present study is that the number of asymptomatic individuals according to the IGRA results was relatively small; therefore, larger-scale studies are necessary to elucidate the role of MBL in various stages of TB.

In addition, differences in bacterial strains should be considered as some studies have reported associations between human polymorphisms in patients with TB and particular *Mtb* strains [39]. Furthermore, the binding of MBL to *Mtb* can be different among *Mtb* strains. A nonfunctional haplotype of a population in Ghana (*LYQC*) is associated with protection against TB caused by *Mycobacterium africanum*, which was identified in 30% of their isolates [8]. The *Mtb* Euro-American lineage accounted for 65% of their isolates, and they did not find any association between *MBL2* genotype and TB caused by *Mtb*. The distribution of *Mtb* lineages is different between Africa and Asia [24], and the Beijing genotype, which was

<4% in the Ghanaian study, was identified in 58% of our Vietnamese isolates [16]. In our study, *YA/YA* was less frequently found in both younger patients with TB caused by the Beijing genotype strain and those with TB caused by the non-Beijing genotype strain, whereas diplotypes with low or deficient MBL levels tended to be more frequently found only in younger patients with the Beijing genotype strain. Although we should be cautious in interpreting the results of subgroup analysis, we speculate that human *MBL2* nonfunctional or low-producing alleles (*B*, *C*, *D*, and *X*) originally evolved to dampen phagocyte-mediated spreading of intracellular pathogens, including the ancestral type of *Mtb* such as *M. africanum*, that may have exploited binding to MBL [40]. However, the recently expanding modern *Mtb* strains, such as the Beijing genotype strains, may have further evolved to be unaffected by MBL deficiency or to take advantage of low MBL levels. It is postulated that 'modern' *Mtb* such as Beijing strain is more pathogenic than other 'ancient' lineages, and that infection with modern *Mtb* progresses faster to active disease presumably because it elicits weaker innate immunity and poorer defense mechanism [41]. In this context, lower MBL levels may facilitate the early progression to active disease caused by the 'modern' *Mtb*. To explain the controversial results from studies on the association between *MBL2* and TB, a study design collecting both host and bacterial information is required because the outcome of TB infection and disease depends on interactions between host and pathogen genotypes [12].

In conclusion, *MBL2 YA/YA*, involved with high levels of plasma MBL, played a protective role against the development of TB in younger (mean age = 32) patients in Viet Nam. Further studies are required to fully elucidate the role of MBL in *Mtb* infection and TB development.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2014.06.006.

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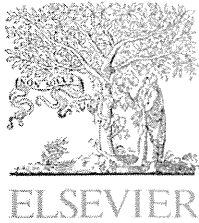
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Association between tuberculosis recurrence and interferon-γ response during treatment

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Summary   *Objectives:* We investigated the relationship between tuberculosis recurrence and *Mycobacterium tuberculosis* antigen-stimulated interferon-gamma (IFN-γ) responses during

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Recurrence;
Interferon-γ release
assay;
Cellular response

treatment.

Methods: Plasma IFN-γ levels in active pulmonary tuberculosis patients (*n* = 407) were analyzed using QuantiFERON-TB Gold In-Tube™ (QFT-IT) at 0, 2, and 7 months of the 8-month treatment received from 2007 to 2009 and the patients were followed up for another 16 months after treatment. Risk factors for recurrence were assessed using the log-rank test and Cox proportional hazard models. Random coefficient models were used to compare longitudinal patterns of IFN-γ levels between groups.

Results: QFT-IT showed positive results in 95.6%, 86.2%, and 83.5% at 0, 2, and 7 months, respectively. The antigen-stimulated IFN-γ responses varied significantly during the treatment course (*P* < 0.0001). Unexpectedly, positive-to-negative conversion of QFT-IT results between 0 and 2 months was significantly associated with earlier recurrence (adjusted hazard ratio, 5.57; 95% confidence interval, 2.28–13.57). Time-dependent changes in IFN-γ levels were significantly different between the recurrence and nonrecurrence groups (*P* < 0.0001).

Conclusions: Although the IGRA response varies individually, early response during the treatment course may provide an insight into host immune responses underlying tuberculosis recurrence.

Introduction

Tuberculosis (TB) remains a major global health problem, resulting in 8.7 million new cases and 1.4 million deaths annually, and multidrug-resistant TB occurs in approximately 3.7% new cases and 20% previously treated cases.[1] Recurrence is thus a major risk factor for multidrug-resistant TB cases[2] and increases the TB burden.[3–5] TB recurrence is defined as a second episode of active disease as a result of relapse (endogenous reactivation) or exogenous reinfection after completion of previous treatment.[6] Biomarkers are necessary for the assessment of treatment effectiveness including early recurrence.[7]

The interferon-gamma (IFN-γ) release assay (IGRA) is an immunological diagnostic test designed to detect TB infection. In this assay, IFN-γ levels produced by primed blood lymphocytes after stimulation with *Mycobacterium tuberculosis* (MTB)-specific antigens in vitro are measured. According to the assay's principle and research findings obtained from animal models, the IGRA response may be attenuated proportional to decreased bacterial antigen load as a result of successful anti-TB treatment.[8,9] However, clinical researchers argue that little correlation exists between the commercial IGRA response and bacillary burden,[10] on the basis of various tests including grade of sputum smear or presence of cavities on chest X-rays (CXRs).[11]

Although many studies have demonstrated a decrease in IFN-γ values during treatment,[12–16] others have shown inconsistent changes and increases have also been reported occasionally.[17–20] Thus, most clinicians believe that monitoring changes in the IGRA response during anti-TB treatment may have limited use in evaluating the effectiveness of treatment[21]; however studies on the relationship of the IGRA response to subsequent episodes of TB recurrence are lacking. In this study, we investigated whether longitudinal patterns of the IGRA response during the treatment period are associated with TB recurrence.

Materials and methods

Ethics statement

A written consent was obtained from each participant. In the case of minors, the parents provided the written consent. The study was approved by the ethical committees of the Ministry of Health, Vietnam and National Center for Global Health and Medicine, Japan.

Study population

In total, 506 unrelated patients aged ≥16 years with smear- and culture-positive pulmonary TB and without history of TB treatment, were consecutively recruited from July 2007 to March 2009 in Hanoi, Vietnam. The MTB culture test was performed using Löwenstein–Jensen media. MTB isolates were subjected to niacin and drug susceptibility tests for streptomycin (SM), isoniazid (INH), ethambutol, and rifampicin. Peripheral blood samples were obtained at diagnosis before initiation of anti-TB treatment (0 months, baseline) for analyzing total blood count, human immunodeficiency virus (HIV) status, and IGRA. IGRA test was repeated at 2 months immediately after the intensive treatment period and at 7 months at the final stage of the maintenance treatment period of the standard 8-month regimen of 2SHRZ/6HE, which was commonly administered during the study period in Vietnam. CXRs were obtained at the baseline and results were interpreted by two unbiased readers blinded to the IGRA results. In the present analysis, patients with multidrug-resistant TB as well as HIV coinfection were excluded.

Follow-up and definitions

During treatment, culture tests were repeated when smear tests were confirmed positive at 2, 5 or 7 months. During the 16-month post-treatment follow-up, sputum smear and culture tests were performed at 2, 4, 7, 10, and 16 months for all accessible cases.

Treatment failure was defined based on the WHO Global Tuberculosis Report in 2012,[1] when the smear and culture were positive at $\geq$5 months or when the smear was positive but culture was not performed, clinical and/or CXR findings indicated failure, and category switched to category II of anti-TB treatment.

Recurrence was defined when patients were cured after treatment, and then suffered from the second TB episode. The second episode was bacteriologically confirmed if the sputum culture was positive at the time of recurrence or the smear was positive or the culture revealed <5 colonies, clinical and/or CXR findings indicated recurrence, and category switched to category II anti-TB treatment.

Interferon-gamma release assay

An enzyme-linked immunosorbent assay-based IGRA kit, QuantiFERON-TB Gold In-Tube™ (QFT-IT; Cellestis, Victoria, Australia), was used for analysis. The guidelines for algorithm and software (QuantiFERON-TB Gold Analysis Software, version 2.50; Cellestis) provided by the manufacturer were followed for the interpretation of results. The testing procedure was carefully monitored as described earlier,[22] and test quality control was performed during each run according to the manufacturer's instructions. When IFN-γ values of negative control "Nil" and positive control "Mitogen-Nil" fell within the appropriate range, the QFT result was assessed as positive when IFN-γ value of "TBAg-Nil" was above the cutoff value (0.35 IU/ml) and negative when the value was below the cutoff value. A positive-to-negative change of QFT results was designated as "negative conversion" in this study.

Measurement of cytokines and chemokines in QuantiFERON-TB Gold In-Tube™ samples

Cytokines and chemokines released in QFT-IT plasma supernatants were collected before treatment and at 2 months and 7 months after the initiation of treatment, from 10 randomly selected recurrence patients and 10 age- and sex-matched nonrecurrence patients were measured using Bio-Plex multiplex system with a 27-plex cytokine-bead kit (Bio-Plex Pro Human Cytokine 27-plex Assay; Bio-Rad Laboratories Hercules, CA). Only values within the asymptotic range were calculated using the standard curve for statistical analysis.

Statistical analysis

Chi square or log-rank tests were used to compare the incidence of recurrence (events) between groups. Influence of time course on the proportion of IGRA-positive results was assessed using generalized estimating equations. Wilcoxon's rank-sum test was used to compare nonparametric distributions between groups. A logistic regression model was used to investigate risk factors involved in treatment failure. The log-rank test of equality across strata and Cox models after testing the proportional hazard assumption were used to assess risk factors for recurrence. The random coefficient model was used to assess influence of time course on the IGRA response and the post-estimation Wald test was used to compare the longitudinal patterns of the response between recurrence and nonrecurrence groups. Bonferroni's correction was applied to correct multiple comparisons. When the IFN-γ value was greater than 10.00 IU/ml, statistical analysis was performed in both two conditions, using a truncated value (10.00 IU/ml) or a value based on extrapolation. Truncated values are presented in parenthesis along with those based on extrapolation when appropriate. The statistical results confirmed that all significant differences found here were demonstrated in both conditions. P values of <0.05 were considered statistically significant unless otherwise specified. Statistical analysis was performed using Stata version 11 (StataCorp, College Station, TX).

Results

Characteristics of the study population

The characteristics of 506 patients recruited have been reported elsewhere.[23] In the present study, we analyzed 407 patients who were enrolled in the directly observed treatment, short-course (DOTS) program at various study sites and did not have multidrug-resistant TB or HIV coinfection at the time of initial diagnosis. Adherence to anti-TB therapy was supervised by the healthcare staff, in cooperation with the patients' family members under the DOTS strategy of the national TB control program. Out of these
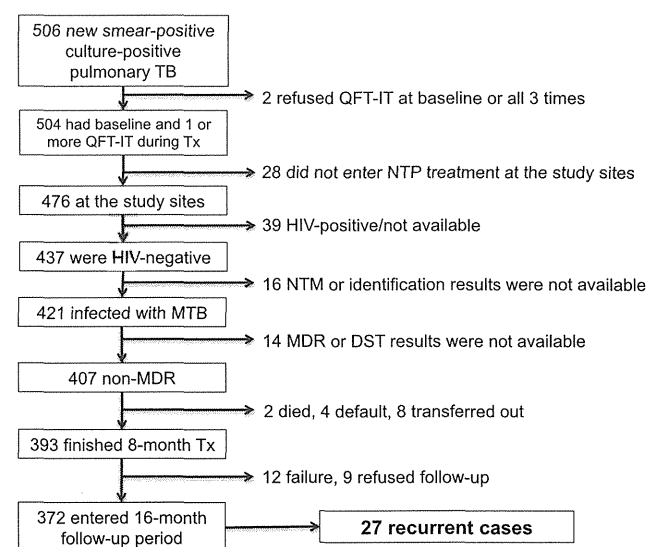

Figure 1   Study flow. TB: tuberculosis; QFT-IT: QuantiFERON-TB Gold In-Tube; NTP: National tuberculosis program; NTM: nontuberculous mycobacterium; MDR: multi-drug resistance; DST: drug sensitivity test; MTB: *Mycobacterium tuberculosis*; Tx: treatment.

Table 1 Patterns of qualitative QFT-IT results during the treatment course (n = 407).

QFT-IT pattern[a]		n	% (95% CI)
Positive-to-Positive-to-Positive	(PPP)	265	65.1 (60.3–69.7)
Positive-to-Positive-to-Negative	(PPN)	27	6.6 (4.4–9.5)
Positive-to-Positive-to-others	(PP_)	40	9.8 (7.1–13.1)
Positive-to-Negative-to-Positive	(PNP)	14	3.4 (1.9–5.7)
Positive-to-Negative-to-Negative	(PNN)	12	2.9 (1.5–5.1)
Positive-to-Negative-to-others	(PN_)	5	1.2 (0.4–2.8)
Negative-to-Negative-to-Negative	(NNN)	8	1.9 (0.9–3.8)
Others		36	8.8 (6.3–12.0)

QFT-IT: QuantiFERON TB-Gold In-tube; TB: tuberculosis; 95% CI: 95% confidence interval.

[a] QFT-IT was performed three times: before treatment, two months, and seven months after starting anti-tuberculosis treatment.

407 patients, 393 completed the 8-month standard treatment course; 381 (97.0%) were cured and 12 (3.0%) did not show treatment response (12/393). Among the cured patients, 372 (97.6%) entered the 16-month post-treatment follow-up (Fig. 1).

The median age of the 372 follow-up patients was 39.7 years (Interquartile range or IQR, 29.0–50.1); 77.7% (289/372) were male, and 238 (64.0%) were current or ex-smokers. Of the MTB isolates tested, 22.8% (85/372) displayed INH resistance with or without SM resistance, and 66.7% (248/372) were sensitive to all 4 major anti-TB drugs tested (data not shown). During the follow-up period, 27 patients (7.3%) showed recurrence.

QuantiFERON-TB Gold In-Tube™ results during treatment period

QFT-IT results were positive in 95.6% (389/407), 86.2% (337/391), and 83.5% (294/352) of the patients tested at 0, 2, and 7 months, respectively, after treatment onset. The proportion of positive IGRA responses varied significantly during the treatment course (P < 0.0001). The proportion of negative conversion (positive-to-negative; PN) between 0 and 2 months, 0 and 7 months, and 2 and 7 months

were 7.9% (31/391), 12.2% (43/352), and 8.4% (29/347), respectively. The patterns of QFT-IT results as measured at the three time points during the course of the treatment period are shown in Table 1.

QuantiFERON-TB Gold In-Tube™ interferon-gamma values during treatment

The median values of IFN-γ, "TBAg-Nil" at 0, 2, and 7 months were 7.33 [IQR 2.53–14.53 (10.00)], 3.22 (1.03–9.54), and 2.54 (0.77–7.80) IU/ml, respectively. IFN-γ values significantly varied during the treatment course (P < 0.0001).

QuantiFERON-TB Gold In-Tube™ results and recurrence

The overall proportion of recurrence was significantly higher in the PN (between 0 and 2 months) group than in the positive-to-positive (PP) group [7/27 (25.9%) vs. 18/311 (5.8%), P = 0.0001] (Table 2). The 1-year recurrence rate was also significantly higher in the PN (between 0 and 2 months) group than in the PP group {25.9% [95% confidence interval (CI), 13.3–46.8] vs. 5.5% [95% CI, 3.4–8.7]}. The log-rank test

Table 2 Proportion of treatment failure and recurrence in TB patients showing positive-to-positive and positive-to-negative patterns of QFT-IT results.

Patterns of QFT-IT results	Treatment failure n/N (%)	P value[a]	Recurrence n/N (%)	P value[a]
Between month 0 and month 2				
Positive-to-Positive	8/325 (2.5)	0.203	18/311 (5.8)	0.0001
Positive-to-Negative	2/30 (6.7)		7/27 (25.9)	
Between month 0 and month 7				
Positive-to-Positive			19/285 (6.7)	0.222
Positive-to-Negative			5/43 (11.6)	
Between month 2 and month 7				
Positive-to-Positive			15/264 (5.7)	>0.999
Positive-to-Negative			1/29 (3.5)	

TB: tuberculosis; QFT-IT: QuantiFERON-TB Gold In-Tube.

[a] By Chi square or Fisher's exact test; comparisons were made between the two groups with different patterns of QFT-IT results; Positive-to-Positive and Positive-to-Negative.
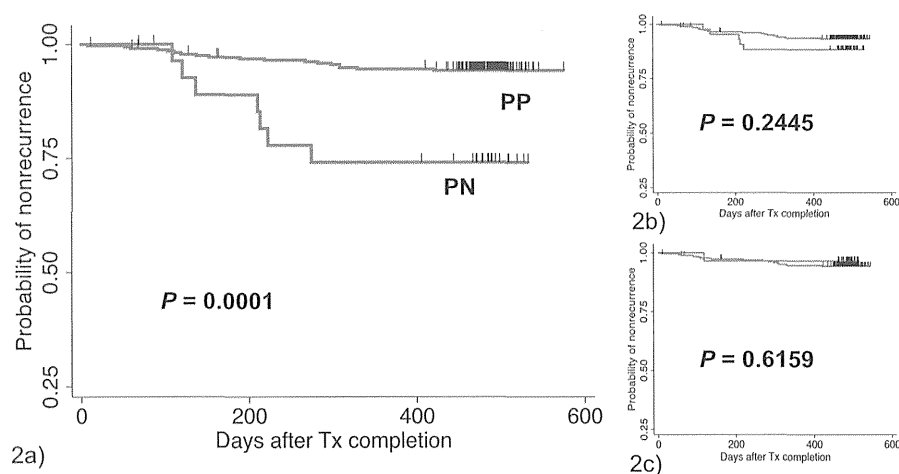
Figure 2 Kaplan—Meier plots stratified by the conversion of QFT-IT results between 0 and 2 months (2a), 0 and 7 months (2b), and 2 and 7 months (2c). QFT-IT: QuantiFERON-TB Gold In-Tube; Tx: treatment; Blue line: positive-to-positive (PP) QFT-IT results. Red line: positive-to-negative (PN) QFT-IT results. The P values were obtained by the log-rank test.

confirmed the difference between the two groups ($P = 0.0001$; Fig. 2a), whereas the conversion of QFT-IT results between 0 and 7 months and between 2 and 7 months did not affect recurrence ($P = 0.2445$ and $P = 0.6159$, Fig. 2b and c, respectively). Among the 27 recurrence cases, MTB isolates were sensitive to all drugs tested in 14 cases (51.9%). INH resistance with or without SM resistance was seen in 8 cases (29.6%). This percentage was slightly higher than that of the nonrecurrence group (77/345 or 22.3%), but the difference was not statistically significant ($P = 0.189$, data not shown). The proportion of this drug resistance was also not different between groups with and without the negative conversion (7/31 or 22.6% vs. 83/332 or 25.0%, $P = 0.919$) (data not shown). Using the Cox proportional hazard model, the

association between recurrence and the negative conversion of QFT-IT results between 0 and 2 months remained significant (hazard ratio, 5.57; 95% CI, 2.28–13.57) after adjusting for BMI at baseline, smear results at 2 months, drug resistance, and smoking status in the final model (Table 3).

QuantiFERON-TB Gold In-Tube™ interferon-gamma values and recurrence

We further assessed possible changes in the actual IFN-γ values using a random coefficient model with log-transformed IFN-γ values of "TBAg-Nil" set as an outcome variable and time of testing, recurrence status, and the

Table 3 Multivariate analysis using Cox proportional hazard model[a] to assess risk factors for recurrence ($n = 372$).

	Proportion (%)	Hazard ratio	95% CI
QFT-IT status at baseline to 2 months after starting treatment			
Positive-to-Positive	18/311 (5.8)	Reference	—
Positive-to-Negative	7/27 (25.9)	5.57	2.28–13.57
BMI		0.86	0.71–1.04
Result of sputum smear at 2 months after starting treatment			
Negative	22/326 (6.8)	Reference	—
Positive	5/46 (10.9)	2.28	0.84–6.16
Drug resistance profile			
Sensitive to all 4 drugs tested[b]	14/248 (5.7)	Reference	—
INH resistance (± SM resistance)	8/85 (9.4)	1.66	0.65–4.19
Other resistant patterns	5/39 (12.8)	2.77	0.96–8.06
Smoking status			
No	8/134 (6.0)	Reference	—
Yes[c]	19/238 (8.0)	1.48	0.61–3.61

95% CI: 95% confidence interval; QFT-IT: QuantiFERON-TB Gold In-Tube; BMI: body mass index; INH: isoniazid; SM: streptomycin.
  [a] Initial model included BMI, sex, age, status of QFT-IT results at baseline to 2 months after starting treatment, presence of cavity or extension of infiltrate on chest radiograph, the results of smear testing at 2 months, patterns of drug resistance, and smoking status. Variables showing $P > 0.2$ were removed from the final model.
  [b] The drugs INH, SM, rifampicin, and ethambutol were tested.
  [c] Current or ex-smokers.

Table 4 Analysis of time-dependent change of interferon-γ values during treatment period using random coefficient model.

	Coefficient	P value	95% CI
TBAg—Nil (log-transformed values) as outcome variable[a]			
Month 2	−0.64	<0.001	−0.74 to −0.54
Month 7	−0.96	<0.001	−1.09 to −0.82
Recurrence	−0.17	0.535	−0.70 to 0.36
Interaction term between 2 months and recurrence	−0.83	<0.001	−1.20 to −0.46
Interaction term between 7 months and recurrence	−0.17	0.513	−0.67 to 0.33
Constant	1.73	<0.001	1.58 to 1.87
Mitogen—Nil (log-transformed values) as outcome variable[a]			
Month 2	0.43	<0.001	0.26 to 0.59
Month 7	1.05	<0.001	0.88 to 1.22
Recurrence	−0.04	0.902	−0.61 to 0.54
Interaction term between 2 months and recurrence	0.22	0.465	−0.37 to 0.81
Interaction term between 7 months and recurrence	−0.04	0.904	−0.64 to 0.57
Constant	1.12	<0.001	0.96 to 1.28

95% CI: 95% confident interval; TBAg—Nil: tuberculosis-specific antigen values minus Nil values; Mitogen—Nil: mitogen values minus Nil values.
[a] 0 month and nonrecurrence group are reference categories.

interaction between the two as independent variables. IFN-γ levels differed significantly with time between the recurrence and nonrecurrence groups according to the post-estimation Wald test ($P < 0.0001$) (Table 4). Fig. 3 shows the linear prediction lines of nonrecurrence and recurrence groups, being overlaid on the basis of individual changes in IFN-γ values.

Statistical significance in the overall difference in QFT-IT IFN-γ values between the two groups prompted us to characterize further estimates. Among the three time points measured, IFN-γ values at 2 months were significantly lower in the recurrence group than in nonrecurrence group {1.36 [IQR, 0.25—3.15] vs. 3.82 [1.12—10.51 (10.00)] IU/ml, $P = 0.003$}, whereas IFN-γ values at 0 months were not significantly different between the recurrence and nonrecurrence groups ($P = 0.1467$). In addition, magnitudes of IFN-γ level changes measured at two consecutive points of time were divided equally into three levels for comparing recurrence time. This indicated that increase of IFN-γ values between 2 and 7 months was significantly associated
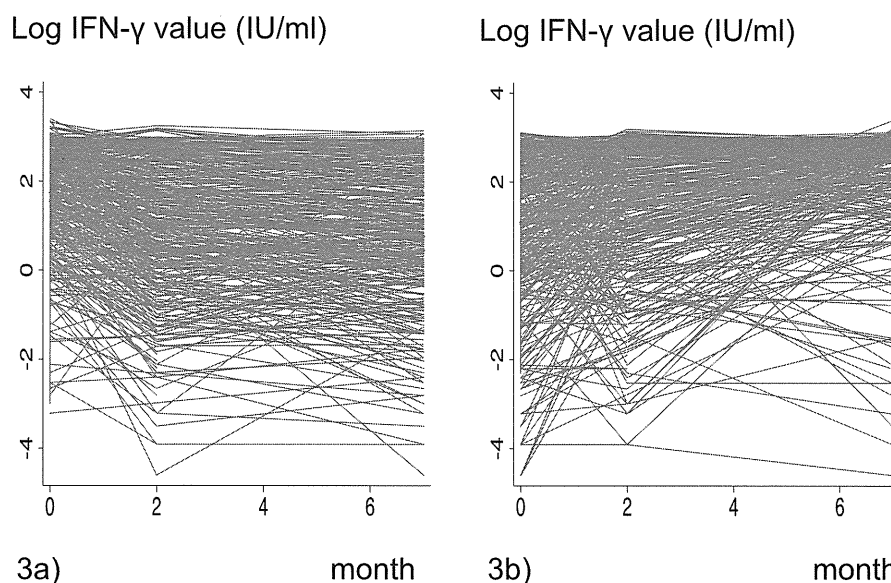

Log IFN-γ value (IU/ml)   Log IFN-γ value (IU/ml)

3a)   month   3b)   month

Figure 3 Linear prediction of transition patterns of interferon-γ that responded to TB-specific antigens (3a) and to mitogen (3b) among recurrence and nonrecurrence cases. TB: tuberculosis; IFN-γ: interferon-γ; Blue line: individual IFN-γ pattern; Upper red line: linear prediction line of nonrecurrence group; Lower red line: linear prediction line of recurrence group.
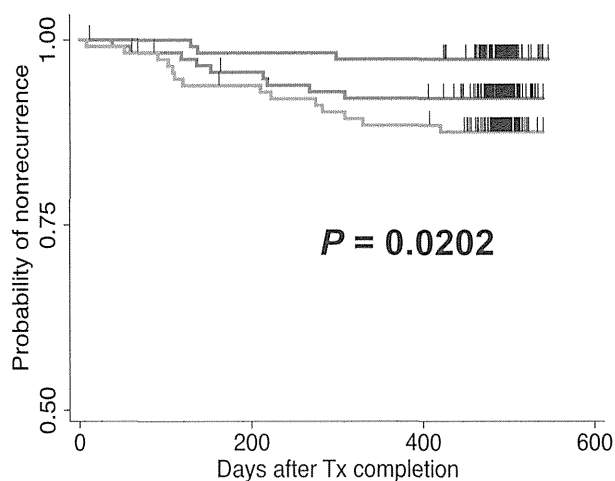
Figure 4 Kaplan—Meier plots stratified by the magnitude of increase in interferon-γ values that responded to TB-specific antigens. The magnitude of increase in interferon-γ values between 2 and 7 months was divided equally into three levels: small (blue line), medium (red line) and large (green line); TB: tuberculosis; Tx: treatment; The P value was obtained by the log-rank test.

with TB recurrence (P = 0.0202). Kaplan—Meier plots are shown in Fig. 4.

QuantiFERON-TB Gold In-Tube™ and treatment failure

The proportion of failure was slightly higher in the PN group (between 0 and 2 months) than in the PP group (Table 2), although no significant association was found even after we ran the logistic regression model with treatment failure as an outcome variable, and the status of negative conversion and result of smear testing at 2 months both as independent variables (data not shown). However, similar to the results observed between IFN-γ patterns and recurrence, the IFN-γ values of "TBAg-Nil" at 2 months were significantly lower in the failure group than in the cure group [median = 1.04, (IQR = 0.21—3.44) vs. 3.46 (1.03—9.82) IU/ml, P = 0.0285].

Cytokines and chemokines in QuantiFERON-TB Gold In-Tube™ plasma supernatants after stimulation with tuberculosis-specific antigens

Among the 27 cytokines and chemokines tested, IL-2, IL-1RA, IP-10, and IFN-γ levels were increased after TB-specific antigen stimulation, and the levels were significantly different compared with unstimulated control levels (data not shown). IL-2, IP-10, and IFN-γ levels tended to be lower in the recurrence group than in the nonrecurrence group (Table 5). Difference in IP-10 and IFN-γ levels at 2 months remained significant even after Bonferroni's correction. IL-10 levels were not different between the conditions (Table 5).

Discussion

In our study, >80% patients had a positive IGRA response until treatment completion, although TB-antigen-stimulated IFN-γ values gradually decreased with time. Interestingly, negative-conversion of the IGRA response after 2 months of treatment was significantly associated with early TB recurrence, and longitudinal patterns of the IGRA response during the treatment course were different between the recurrence and nonrecurrence groups.

According to most of the previous studies, the proportions showing positive IGRA responses before, during, and after anti-TB treatment tend to decrease in a time-dependent manner, but are largely variable.[12–15] High proportions of positivity before and during the treatment period in our study may have resulted from strong TB-antigen-specific IFN-γ response before treatment (median IFN-γ levels = 7.33 IU/ml), presumably because of high bacillary burden in immunocompetent individuals. IFN-γ levels are known to not easily decrease below the cutoff level in such cases.[24,25] Frequent exposure to MTB is one of the reasons for relatively high IFN-γ values during treatment course.[26]

Nevertheless, our study showed that IFN-γ values varied and gradually decreased with time. This finding is consistent with earlier reports,[12–15] but varies from other results in which IFN-γ values did not change remarkably[17] or increased.[19] In the Indian study cohort,[17] a large proportion of the subjects were hospitalized as compared with our study (80.0% vs. 22.1%), and the patients may have suffered from more severe disease than those in the present study. In such cases, recovery or elevation of IFN-γ levels may be observed after starting the effective treatment, although the bacterial antigen load may decrease. Such a paradoxical response may be often observed after several days of blood-cell incubation, to allow proliferation of IFN-γ-producing cells, as reported previously.[27]

Negative conversion of the IGRA response after 2 months of treatment was significantly associated with early recurrence in our cohort, even after adjustment for possible confounding factors. This was different from our expectation; no studies have attempted a possible association between actual recurrence and the IGRA response during treatment. TB antigen-specific IFN-γ levels observed at 2 months were also significantly lower in the recurrence group than in the nonrecurrence group. Pretreatment IFN-γ levels were slightly lower in the recurrence group (median = 6.03 IU/ml) than in the nonrecurrence group (median = 7.89), but were considerably higher than the cutoff value. Therefore, frequent negative conversion at 2 months in the recurrence group cannot be attributed to a simple fluctuation of IFN-γ levels around the cutoff value. As a possible confounder, the proportion of INH-resistance was not significantly associated with recurrence or negative conversion in our study, indicating that 2SHRZ/6HE, a previous standard treatment regimen during the study period in this area, did not affect our main findings.

In general, TB recurrence tends to occur when the initial disease course is severe and prolonged.[28] Peripheral blood cells may not sufficiently respond to TB-specific antigens in such a disease state. Notably, suppression of IFN-γ pro-