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

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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) ≦ 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.

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].

Abbreviation: *Mtb*, *Mycobacterium tuberculosis*.

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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).