Invited Review

Serodiagnosis of *Mycobacterium avium* Complex Disease in Humans: Translational Research from Basic Mycobacteriology to Clinical Medicine

Kazuo Kobayashi*

*Sakai City Institute of Public Health, Osaka 590-0953, Japan*

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**SUMMARY:** Rapid and accurate diagnosis of infectious diseases, including mycobacterial disease such as tuberculosis (TB) and diseases due to nontuberculous mycobacteria (NTM), is a very important element of global health. The gold standard in diagnosis of mycobacterial diseases remains clinical examination, combined with direct microscopic examination of sputum and culture of bacteria. Culture of slowly growing mycobacteria, including *Mycobacterium tuberculosis* and NTM (such as *M. avium* complex: MAC), can take up to 4 to 6 weeks, and in 10–20% of cases the bacillus is not successfully cultivated. Diagnosis of MAC pulmonary disease (MAC-PD) is complicated and time-consuming (usually at least 1 month). I have characterized the nature of MAC antigens and immune responses from the aspect of basic mycobacteriology, and then translated to clinical science. My multicenter study in Japan has demonstrated the usefulness of a serodiagnostic test to determine serum IgA antibodies against mycobacterial glycopeptidolipid (GPL) core antigen for diagnosing MAC-PD within a few hours. To validate in a larger number of patients, at diverse geographic locations, and among other races, the test was also assessed the usefulness internationally in the United States and Taiwan. In this review, I discuss development of serodiagnosis of MAC-PD by translational research and international collaboration study.

1. Introduction

Mycobacterial diseases, such as tuberculosis (TB) and nontuberculous mycobacterial disease, continue to pose major public health challenges in developed, as well as developing, countries. Among mycobacterial diseases, pulmonary disease due to nontuberculous mycobacteria (NTM) has been increasing recently, although the incidence of TB has been falling worldwide for about a decade (1,2). In Japan and the United States of America (USA), *Mycobacterium avium* complex (MAC) accounts for approximately 70–80% as a causative agent of NTM disease (1,3). The annual incidence rate of NTM diseases per 100,000 people is approximately 6 in Japan (4). MAC is now widely recognized as an important pathogen that causes chronic and progressive pulmonary disease (PD) even in patients without human immunodeficiency virus (HIV) infection. The increasing incidence of MAC pulmonary disease (MAC-PD) makes its diagnosis and management a matter of increasing concern to respiratory physicians.

The diagnosis of MAC-PD is difficult because in contrast to *Mycobacterium tuberculosis*, MAC contamination of clinical specimens can come from environmental sources such as water, dust and soil, and it may colonizes the respiratory tract without any accompanying invasive disease (1). The diagnosis rests on recovery of the pathogen from cultures, although sputum cultures positive for MAC do not in themselves prove infection because MAC may exist as saprophytes in the airway or as environmental contaminations. Thus, isolation of MAC from sputa is insufficient to document pulmonary infection. According to the guideline of the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) (1), the diagnosis of PD due to MAC is complicated and time-consuming. MAC is ubiquitous in nature and the diagnosis of MAC diseases is required for clinical and microbiologic findings, such as repeated isolation/cultures of MAC from sputa (Table 1). In addition, it is also difficult to discriminate MAC-PD from infection due to other mycobacteria in the absence of culture results, because clinical features such as symptomatic or radiographic findings are very similar in mycobacterial
diseases. There are no known instances of human-to-
human transmission of NTM and therefore the isolation
of patients is unnecessary. From the aspect of infection
control, it is particularly important to distinguish be-
 tween MAC-PD and pulmonary TB. In light of the
difficulties in obtaining a clear-cut diagnosis of MAC-
PD, particularly given the propensity for this disease to
afflict relatively elderly and frail patients, less invasive
diagnostic methods are needed.

To overcome these difficulties, development of rapid,
simple, noninvasive, and accurate methods to diagnose
MAC disease is needed to control the disease. In this
regard, serodiagnosis that differentiates TB and disease
due to *M. kansasii* and MAC is required, because MAC,
but not the majority of *M. tuberculosis* and *M. kansasii*,
is resistant to most of the available anti-TB drugs (1).
We have developed enzyme immunoassay (EIA) for
serodiagnosis of active MAC disease using glycopep-
tidolipids (GPLs) that are the major and specific cell
surface antigens of MAC. The EIA is approved by the
Ministry of Health, Labour and Welfare of Japan and
now commercially available in clinical use. In addition,
it is also assessed internationally in USA and Taiwan. In
this review, I discuss development of serodiagnosis of
MAC-PD by translational research from basic
mycobacteriology to clinic and international collabora-
tion study to serodiagnose and control the disease.

2. GPL antigens of MAC

2–1. Chemical structure

Figure 1 shows the features of major MAC antigen,
GPL used in the immunoassay. The chemical structure
of GPL is composed of a common GPL core, fatty acyl-
D-Phe-D-allo-Thr-D-Ala-L-alaninol-O-(3,4-di-
O-methyl-
Rha), and the different moiety of oligosaccharide linked
at the Thr of the core (3,5). The core is composed of fatty
acid, 3 amino acids, and rhamnose. GPLs are the
major and specific cell surface antigens of the MAC and
*M. scrofulaceum* group, and they can be subdivided
into 31 distinct serotypes based on the oligosaccharide.
The approximate MW of GPL core is 1.2 kilodalton
(5–7).

Besides MAC, *M. scrofulaceum*, *M. chelonae*, *M. ab-
scessus*, and *M. fortuitum* also possess GPL antigens in
their cell walls (3,5,8). Because *M. chelonae*, *M. abces-
sus*, and *M. fortuitum* are rapidly growing mycobacte-
ria, diseases due to such mycobacteria are rapidly diag-
nosed. By contrast, slowly growing *M. kansasii* and *M.
tuberculosis* complex, including BCG, do not have GPL
(Table 2).

2–2. Biologic roles of GPL

GPL is involved in colony morphology, hydrophobic-
ity and biofilm formation of the bacterium (3). In the
infected host, GPL prevents phagocytosis by macro-
phages, inhibits phagosome-lysosome fusion, and
cytokine production via through toll-like receptor
(TLR) 2 pathway. Thus, GPL exerts a pleiotropic activi-
ty on both bacteria and hosts (3,7).

3. Development of serodiagnosis of active MAC-PD

3–1. Antigenic epitope: GPL core

My early study of the serodiagnosis, I used a mixture
of GPL antigens derived from 11 distinct serotypes of
MAC (8). However, there are problems with the transi-
tion of the assay from a research tool to widespread
clinical use. Specifically, the preparation of GPL anti-

![Fig. 1. Chemical structure of mycobacterial glycopeptidolipid (GPL).](image)

GPL core is a common in all serotypes of mycobacteria possessing GPL indicated by broken lines (–).
gen that is of a consistent quality, as well as quantity, from 11 reference strains of MAC is both time- and cost-consuming. Identification of a simple and stable antigen for use in serodiagnostic tests for MAC disease is necessary. The titers of GPL antibody were reduced to background levels by adding GPL core antigens and antibody activity was absorbed by them. As a result, the immunodominant epitope is GPL core (9), which is common in all serotypes of mycobacteria possessing GPL.

3–2. Antibody class

From the aspect of sensitivity and specificity of EIA detecting serum antibodies specific for MAC-GPL, I next determined antibody class, such as IgG, IgA, and IgM. The respective sensitivities and specificities of GPL core-based EIA for diagnosis of MAC-PD were 72.6% and 92.2% for IgG, 92.5% and 95.1% for IgA, and 78.3% and 91.0% for IgM when compared to the gold standard, the diagnostic criteria of ATS and IDSA (1) (Table 1). The best sensitivity and specificity for the diagnosis of MAC-PD was obtained by measuring IgA antibodies against GPL core (9).

3–3. Serodiagnosis of active MAC-PD proven by repeated sputum cultures

My colleagues and I have attempted the serodiagnosis of active MAC-PD, when compared to MAC contamination, TB, NTM diseases (M. kansasii and M. fortuitum) rather than MAC, other lung diseases including chronic obstructive pulmonary disease, interstitial pneumonia, cancer, bacterial pneumonia and sarcoïdosis, and healthy subjects (10,11). A six-institution multicenter study has demonstrated sensitivity; 84% and specificity; 100% for the serodiagnosis of active MAC-PD (11) proven by repeated sputum cultures when cut-off level of IgA antibody against GPL core was set at 0.7 U/ml (Figure 2). The serodiagnosis can differentiate MAC disease from disease due to M. kansasii, MAC contamination, TB, chronic obstructive pulmonary disease, interstitial pneumonia, cancer, bacterial pneumonia and sarcoïdosis, but not disease caused by M. fortuitum possessing GPL (12).

3–4. Serodiagnosis of active MAC-PD proven by bronchial wash culture using bronchoscopy

MAC-PD is usually diagnosed according to the guidelines set forth by the ATS/IDSA, which include clinical and microbiological criteria (1), as mentioned previously. Bronchoscopy to obtain bronchial wash for culture is often considered in patients in whom MAC-PD is difficult to diagnose by routine sputum examination; however, it is difficult to perform bronchoscopy in all patients, because bronchoscopy is invasive. In light of the difficulties in obtaining a clear-cut diagnosis of MAC-PD, particularly given the propensity for this disease to afflict relatively elderly and frail patients, less invasive diagnostic methods are needed. The novel approach to help diagnose such cases has been needed. I have demonstrated sensitivity; 79% and specificity; 96% using the serodiagnosis for active MAC-PD proven by bronchial wash culture (12). The result indicates the availability of serodiagnosis of MAC disease proven by bronchoscopy, although both sensitivity and specificity were slightly low when compared to such disease with sputum-positive MAC-PD.

4. International collaboration studies of the serodiagnosis

Obviously the serodiagnosis by EIA requires validation in a larger number of patients, at diverse geographic locations, and among other races. To extend the usefulness of EIA kit for MAC-GPL internationally, I have compared cut-off level, sensitivity and specificity among Japan, USA, and Taiwan (Table 3). The performance in Taiwan (sensitivity: 61%, specificity: 91%) (13) was relatively low when compared to Japan (sensitivity: 84%, specificity: 100%) (11) and USA (sensitivity: 77%, specificity: 94%) (14). Possible reasons for low sensitivity and specificity in Taiwan include relatively high incidence of diseases due to rapidly growing mycobacteria possessing GPL and study population contains immunocompromised hosts, although studies in Japan and USA employed sera of patients with im-

![Fig. 2. The level of serum IgA antibody to GPL core antigen. Antibody levels in MAC-PD are significantly higher than in the other groups. Horizontal broken line indicates the cut-off value (0.7 U/ml).](image-url)
munocompetent MAC-PD. Similarly relatively low sensitivity (77.5%) obtained from the South Korea in where disease due to rapidly growing mycobacteria possessing GPL was common (15), although the serodiagnostic performance differentiating MAC-PD and TB is high (100%). I used the cut-off value at 0.7 U/mL in both Japan (11) and Taiwan (13), but 0.3 U/mL was set in the USA (14) based on the best performance calculated by the receiver operating characteristic curve obtained each study. As a result, the cut-off value in the US was low compared to Japan and Taiwan.

5. Conclusions and future perspectives

My colleagues and I have developed a promising, rapid, noninvasive test that has potential to aid in deciding whether or not a sputum smear containing acid fast bacilli is due to TB, contamination, or MAC. The EIA kit (Capilia MAC Ab ELISA, TAUNS Laboratories, Shizuoka, Japan) for serodiagnosis of active MAC disease has become commercially available for clinical use in Japan. Besides active MAC-PD, the test requires availability in extrapulmonary MAC disease, latent MAC infection and hypersensitivity lung disease (hot tub lung disease) due to MAC. Hopefully the test will be included in official diagnostic criteria of ATS/IDSA in the near future. Consequently, the serodiagnosis by detecting serum antibodies to MAC-GPL helps control the disease.

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Conflict of interest None to declare.

REFERENCES


Table 3. Comparison of serodiagnostic performance among Japan, USA, and Taiwan

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<th>Cutoff (U/mL)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<td>100</td>
<td>Am J Respir Crit Care Med. 2008 (11)</td>
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<td>Taiwan 0.7</td>
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<td>PLoS One. 2013 (13)</td>
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<td>USA 0.3</td>
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