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Potential of multi-component antigens for tuberculosis diagnosis

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A B S T R A C T

Tuberculosis is one of the top ten causes of deaths worldwide. The cause of tuberculosis is a bacterium, *Mycobacterium tuberculosis*, which has been surviving for centuries. Immunological tests based on detecting the presence of antibodies in the sera of active TB patients against various antigens of *M. tuberculosis* are useful for diagnosis of TB and offer simple, rapid and cost effective methods most suitable for poor and developing countries. Several recombinant antigens have been reported so far with varying sensitivity individually, yet none had shown sensitivity higher enough to be used in a commercial test. There is a trend of utilizing recombinant DNA technology to make polypeptide chain with two or more different antigenic regions, in order to increase the diagnostic efficiency. In this review, we have made an attempt to combine current studies on the usefulness of the multi-component *Mycobacterium tuberculosis* antigens in serological tests for the diagnosis of tuberculosis.

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1. Introduction

Tuberculosis (TB) is the cause of more than one million deaths worldwide. The emergence of drug resistant strains is raising concerns at the global level and prompted health authorities to strengthen and reinforce control strategies to limit their spread.

A rapid, reliable and cheap procedure for diagnosis of tuberculosis has remained the cornerstone of controlling and treatment of the disease. Several methods including isolation of *Mycobacterium tuberculosis* (*M.tb*) from sputum sample, sputum smear microscopy, chest X-ray, serodiagnosis or detection of bacteria through molecular methods are available. *M.tb* isolation in bacteriological culture is currently the gold standard for diagnosis of TB. However, culture takes several weeks to confirm a clinical diagnosis and smear microscopy has specificity and sensitivity limitations [30,34]. A new PCR based method, the GeneXpert MTB/RIF (Cepheid Inc., USA) has been endorsed by WHO recently. It is a cartridge-based, automatic test that not only detects TB but also performs rifampicin resistance testing. This assay showed a high sensitivity in both pulmonary [5] and extra pulmonary TB [16]. However, its high cost is a major limitation for its widespread use in areas where the prevalence of the disease is higher [29].

Latent TB patients show no clinical or radiographic symptoms neither could be identified through molecular assays. Tuberculin skin testing (TST) is used sometimes, but it has poor specificity and also patients need to visit again for evaluation. Another test is interferon-γ release assay, that has good sensitivity, yet this assay is technically complex and require several days to process [15,25].

Detection of antibodies or antigens in blood of patient through immunoassay has been a successful diagnostic method in many infectious diseases. Efforts to develop serological tests for identification of *M.tb* infection have been ongoing for many years. However, an immunoassay using single or multiple target antigens is yet to show high enough sensitivity and specificity to meet the requirements for clinical utility.

The specific antibody response in TB patients is intricate and not completely understood. The complexities can be attributed to a number of factors like the evolved relationship of the pathogen with the immune system, *M.tb* intracellular localization, and the partial understanding of *M.tb* biology and its interaction with host. These impediments have restrained the attempts to exploit the host response in antibody detection as an economical diagnostic alternative in low-income populations. In 2011, WHO issued a policy recommendation against the use of the various commercial serological tests for TB diagnosis [33] due their inconsistent sensitivity and specificity values. However, they recommended that further research and development is required in this field.

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The research to identify new protein targets and investigation of different combinations of antigens for developing a reliable serodiagnosis of TB has continued. These efforts have been the subject of several reviews [4,31]. In early studies, antigens purified from bacterial cultures or produced through recombinant DNA technology were evaluated. There are reviews present that compared enzyme-linked immunosorbent assay (ELISA) using different antigens [9,28]. With the advancement in technology, some new strategies to improve sensitivity of existing antigens have been introduced. Such as in one study, a truncated variant of PstS1 that preserved all known B-cell linear epitopes, revealed to increase sensitivity for ELISA based diagnosis of TB from 36% to 43% [22].

This review presents the current status of the application of TB recombinant antigens in the diagnosis of TB, specially on the use of the new generation of diagnostic tools, chimeric antigens, in serological tests.

2. Mixture of recombinant antigens

Genome sequencing, reverse genetics, comparative genomics and proteomics led to identification of a dozen of antigens. Many of these recombinant antigens have been reported for the detection of *M.tb* specific antibodies in human serum samples [19,32]. In most studies, recombinant antigens were alone coated on the surface of ELISA plates. Yet, in some cases, combination of two or three proteins were coated [39]. Most of the cocktails reported were antigenic that detected antibody in the sera of TB patients. However, a well-advised and precise selection of mixture components is crucial to obtain a preparation useful for diagnostic application.

3. Fusion antigens

Recently, there has been a trend in making multi-epitope or fusion recombinant antigen. Use of single polyprotein, instead of mixture of various antigens is potentially cost-effective, especially for developing a test that could be used for developing countries where cost per test is important. The focus of this review is on the performance of fusion antigens in serodiagnosis of tuberculosis. A major advantage of using a recombinant multiepitope antigen for antibody detection rather than the tests based on the mixture of antigens would be a more standardized approach. This new generation of recombinant products has the potential to replace the native antigen(s). There are reports that showed usefulness of fusion antigen (Table 1).

Yang et al. reported a recombinant fusion protein Rv0057-Rv1352 that showed good immunoreactivity with serum from TB patients in western blotting. The authors have reported antibody response in sera of relatively small population of 69 TB patients and 60 patients with non-TB respiratory diseases that were used as control. The sensitivities of a diagnostic ELISA test using Rv0057-Rv1352 fusion was 60.3% and using 38 kDa-16 kDa was 58.8% [37].

Pleural tuberculosis (PL-TB) is a type of extra pulmonary tuberculosis. Araujo et al. reported construction of the fusion of the Rv3019c (MT10.3) and Rv1980c (MPT64) antigens. The fusion was evaluated in 92 pleural fluid (PF) samples in IgA ELISA. Using MT10.3-MPT64 sensitivity of 70% was achieved while the overall sensitivity of histopathological examination, was 74%. Furthermore, the ELISA IgA MT10.3-MPT64 test sensitivity was high for samples with a negative culture (23/27; 85.2%) or nonspecific histopathology (17/18; 94.4%) [3].

A recombinant fusion of three immunodominant antigens 38-kDa-16-kDa-ESAT-6 was constructed and evaluated by Wu et al. Evaluation was done using 105 patients with tuberculosis (TB) and 45 control individuals and found that the sensitivity and specificity for detecting antibody responses to the recombinant antigen were 65.4% and 84.8%, respectively. Authors also evaluated the recombinant 38-kDa-16-kDa-ESAT-6 MTB antigen with commercial ELISA TB-DOT (TB-directly observed therapy) kit and found the fusion antigen more effective in distinguishing between TB patients and controls [35].

Another fusion of three antigens from *M. tuberculosis* Rv2031c (hspX), Rv1980c (mpt64), and Rv0934 (pstS1) was constructed and evaluated in 171 TB patients and 86 controls by ELISA. Compared with the three individual antigens (16 kDa: sensitivity 19.9%, specificity 96.5%; MPT64: sensitivity 75.4%, specificity 34.9%; 38 kDa: sensitivity 33.3%, specificity 83.7%), the fusion protein antigen (sensitivity 42.1%, specificity 89.5%) gave the best diagnostic performance [6].

Although difficult to construct, there are reports of fusion construct that combine more than three proteins. A polyprotein consisting of four proteins, Mtb11 (also known as CFP-10), Mtb8, Mtb48 and 38-kDa. The ELISA test showed sensitivity of 71.5% in Brazilian population and a specificity of approximately 98%. Furthermore, the antigen combination detected a number of HIV-TB co-infections as well [17].

In another study, two fusions, each consisting of three antigens as well as a big fusion combining two polyproteins were made. Two novel *M. tuberculosis* fusion proteins, 38kD-ESAT6-CFP10 (38F) and Mtb8.4-MPT64-TB16.3-Mtb8 (64F), were expressed. Multiple proteins fusion 38F-64F was also constructed and evaluated. The sensitivity of the novel 38F-64F indirect ELISA alone was much higher that of the sputum smear test (78.64% vs. 47.57%). Furthermore 38F-64F polyprotein also showed a sensitivity of 74.16% with sera from extra pulmonary TB patients. The specificity was 90.36%

Table 1
M.tb Chimeric fusions consisting of two or more full-length antigens.

Fusion antigen	Test method	Subjects of ELISA	Serodiagnostic results	Reference
Rv0057-Rv1352	IgG ELISA	69 TB patients, 60 patients with non-TB respiratory diseases	Sensitivity 60.3% Specificity 93.3%	[37]
Rv3019c (MT10.3) and Rv1980c (MPT64)	IgA ELISA	92 pleural fluid (PF) Pleural tuberculosis	Sensitivity 85.2%	[3]
16-kDa-38-kDa-ESAT-6	IgG ELISA	105 patients with tuberculosis (TB), 25 non-TB pulmonary disease patients and 20 healthy individuals	sensitivity 65.4% specificity 84.8%, more effective than the TB-DOT kit	[35]
Rv2031c (hspX), Rv1980c (mpt64), and Rv0934 (pstS1)	Ig G ELISA	171 TB patients, 86 controls	sensitivity 42.1%, specificity 89.5%	[6]
CFP-10, Mtb8, Mtb48 and 38-kDa	ELISA	144 TB confirmed 299 Healthy control	Sensitivity 71.5% Specificity 98.33%	[17]
38kD-ESAT6-CFP10-Mtb8.4-MPT64-TB16.3-Mtb8	ELISA	healthy blood donors non-TB patients	Sensitivity 78.64% specificity 92.3%	[10]

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