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MINI-REVIEW

Skin tests for the detection of *Mycobacterial* infections: achievements, current perspectives, and implications for other diseases

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Abstract

Immunological and molecular advances have modernized diagnostic testing for many diseases. Although interferon gamma-release and polymerase chain reaction assays have been developed to detect *Mycobacterium tuberculosis* (Mtb) infection, purified protein derivative (PPD)–based tuberculin skin testing (TST) remains the most widely used method. Indeed, the TST is a simple and cost-effective tool that can be easily applied for widespread screening for Mtb infection. However, the lack of specificity has been a limitation of these tests, and, more recently, supply issues have arisen. Building upon the skin tests that historically have been used within TB and leprosy control programs, we discuss recent developments using modern technologies for improving mycobacterial skin testing as well as practical advantages inherent to the technique. Furthermore, we outline how this knowledge could be applied to develop similar tests that could benefit diagnostic strategies for other infections.

Key points

- *Skin testing provides a significantly cheaper alternative to most modern technologies.*
- *Skin tests provide a lab-independent diagnostic strategy that can be widely administered.*
- *Diseases for which T cell responses are more robust or durable than antibody responses are accessible for skin testing.*

Keywords Leprosy · Tuberculosis · Skin · T cell · Diagnosis

Introduction

Skin testing is a widely known diagnostic method used in allergy and infectious diseases involving intradermal injection of a small amount of antigen to assess either immediate IgE-mediated allergic or antigen-specific T cell–mediated delayed-type hypersensitivity (DTH), responses. Exemplified by the tuberculin skin test (TST) that was originally created by Robert Koch and developed into the intradermal technique in 1912 by Charles Mantoux, multiple iterations have been used to detect mycobacterial infections and guide disease control efforts.

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Tuberculosis

Estimates are that nearly 2 billion people latently infected with *Mycobacterium tuberculosis*, and 5–10% will progress to TB disease during their lifetime (Getahun et al. 2015; Houben and Dodd 2016; WHO 2018a). Indeed, a now relatively stable number of around 10 million people are sickened with tuberculosis (TB) each year. Among these cases, in 2018, there were an estimated 1.2 million TB-associated deaths among HIV-individuals and a further 0.25 million among HIV-infected people (WHO 2019). This makes TB the leading cause of death from a single infectious agent and propels it into the top 10 causes of death overall. The World Health Organization (WHO) End TB Strategy recognizes that unless *M. tuberculosis* (Mtb) transmission is disrupted, global TB elimination targets are unlikely to be attained (Lonnroth and Raviglione 2016; Uplekar et al. 2015; WHO 2018b). Accordingly, the US Centers for Disease Control and Prevention (CDC) Division of Tuberculosis Elimination's strategic plan includes accelerating the decline in TB through

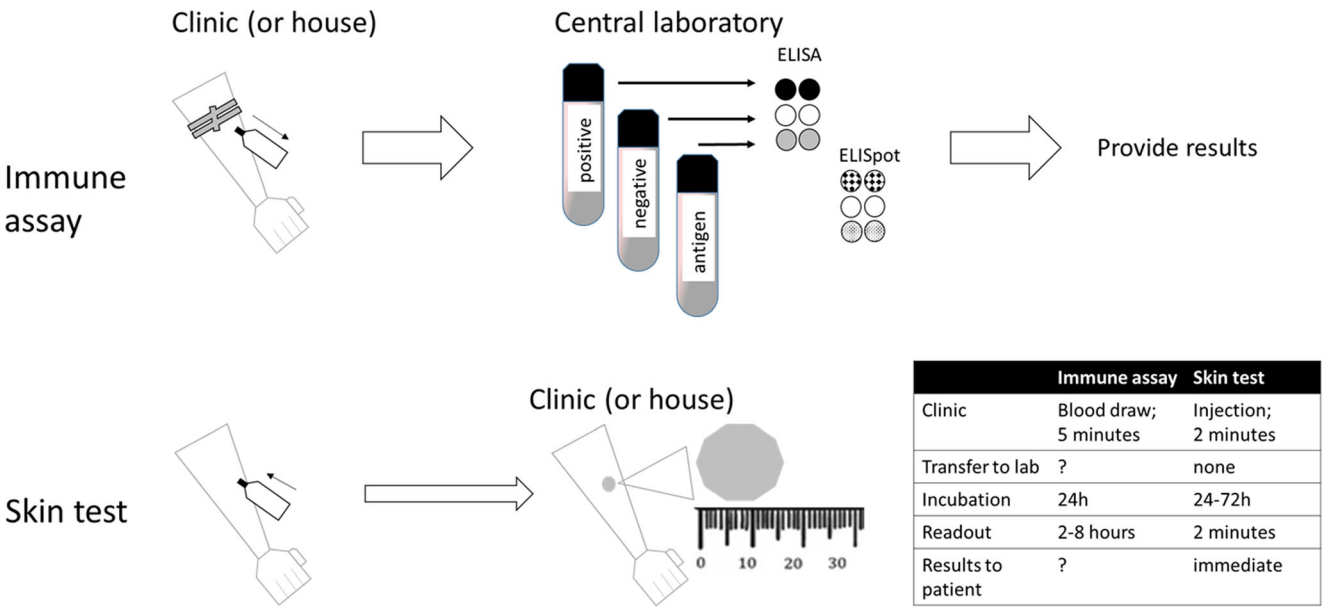
targeted testing and treatment of latent TB infection (LTBI) (CDC 2020).

At present, TB diagnosis uses indirect methods such as chest X-rays a well as direct detection methods that include 4-week culture of Mtb from sputum samples. Nucleic acid amplification tests (NAAT; e.g., GeneXpert) have sped up the provision of more sensitive and specific results (Boehme et al. 2011; Nicol et al. 2011), and IFN-γ release assays (IGRA) that assess cytokine production following incubation of unfractionated blood with Mtb antigens have also been developed (Arenas Miras Mdel et al. 2013; Arias Guillen 2011; Rangaka et al. 2012) (Fig. 1). Clinicians have great familiarity with skin testing, which requires no laboratory-based technology, and TST has been the standard TB screening/surveillance tool for decades (Bass Jr 2003; Snider Jr 1982).

TST uses purified protein derivative (PPD; tuberculin) to detect T cell responses against Mtb. PPD was produced by first steaming cultures of *Mtb* then repeatedly precipitating with ammonium sulfate to purify the proteins while reducing polysaccharide, nucleic acid, and lipid content. In 1944, a large lot of PPD-Standard (PPD-S) was made that comprised approximately 92.9% protein, 5.9% polysaccharide, and 1.2% nucleic acid. Eight years later, the World Health Organization (WHO) adopted the PPD-S as the international standard (Guld et al. 1958; Seibert and Glen 1941). The Food and Drug Administration (FDA) required that all lots of PPD be qualified and show a potency equivalent to PPD-S. A standard preparation derived from one solitary batch, Master Batch Connaught Tuberculin (CT68), was then adopted to eliminate batch-to-batch variation (Landi and Held 1980; Sbarbaro 1978). There are now, however, many varieties of PPD in use: PPD-S in the USA; PPD RT23 is the most widely used PPD product outside of North America; PPD RT23 Mexico is used in Latin America; PPD-s is used in Japan; and PPD-L is used in Russia (Comstock et al. 1964; Kimura et al. 2005; Rangel-Frausto et al. 2001; Starshinova et al. 2018; Yang et al. 2012). PPD-S2, the current US standard that was developed to address the eventual depletion of PPD-S, is used in the commercially available Aplisol® and Tubersol® (Jensen et al. 2005; Villarino et al. 2000). Although results with Aplisol® and Tubersol® were comparable to those of the original PPD-S, shifting the use between Tubersol® and Aplisol® has resulted in aberrations that are unexplained (Gillenwater et al. 2006; Mehta et al. 2009; Villarino et al. 1999).

Both Aplisol and Tubersol have experienced regular shortages (CDC 2013b) and routine health department activities had been threatened or already curtailed in 56% US jurisdictions due to reported shortages of PPD TST antigen solutions (10 Tubersol only, four Aplisol only, and 15 both) (CDC 2013a). In June 2019, the US Centers for Disease Control (CDC) announced an anticipated 3–10-month shortage of Aplisol (2019). This limitation could potentially be overcome through the use of defined, species-specific proteins. Mass spectrometry and molecular analyses identified over one hundred proteins from four different PPD, and revealed that roughly 60% of the total protein content is contributed by heat shock proteins GroEl, GroEs, DnaK, and HspX (Borsuk et al. 2009; Cho et al. 2012). These heat shock proteins are conserved among most mycobacterial species, and it is believed that this broad mixture limits the ability of PPD-based TST to distinguish *Mtb* infection from either exposure to non-tuberculous mycobacteria or vaccination with BCG (Farhat et al. 2006; Huebner et al. 1993).

Many TB experts have favored the development of a skin test that could be more accurate for the differential diagnosis of active



	Immune assay	Skin test
Clinic	Blood draw; 5 minutes	Injection; 2 minutes
Transfer to lab	?	none
Incubation	24h	24-72h
Readout	2-8 hours	2 minutes
Results to patient	?	immediate

Fig. 1 Logistics of conducting ex vivo T cell–based testing and in vivo skin testing

TB and LTBI (Pai et al. 2016; Rangaka et al. 2012; Rose et al. 1995). We previously demonstrated in preclinical assessments that a novel recombinant protein encoded by the *Rv0061* gene that is unique to Mtb had potential as a skin test for Mtb infection. Named DPPD after the first 4 amino acids in the N-terminus sequence, this protein elicited DTH in 100% of *M. tuberculosis*–infected guinea pigs but not in animals sensitized with other representative *Mycobacteria* species (*M. bovis*–BCG, *M. avium*, *M. kansasii*, *M. fortuitum*, *M. gordonae*, *M. chelonae*, *M. scrofulaceum*, *M. smegmatis*, *M. terrae*, and *M. vaccae*) (Coler et al. 2000). Recombinant DPPD produced either in nonpathogenic *Mycobacterium smegmatis* or in *Escherichia coli* as a fusion driven from three tandem DPPD gene copies elicited DTH responses in *M. tuberculosis*–infected guinea pigs that were indistinguishable from those elicited by PPD. (Liu et al. 2004). A proof-of-concept study indicated that intradermal DPPD skin testing detected individuals exposed to Mtb with higher sensitivity and specificity than had previously been reported with PPD (Campos-Neto et al. 2001). In further evaluation of DPPD, irrespective of the TB presentation as cavitary, disseminated, or lymphadenopathic presentation, all HIV-negative active TB patients assessed had indurations > 10 mm following DPPD inoculation. Although there was a highly significant correlation between PPD and DPPD results among TB patients, assessment among participants without active TB but with previous BCG vaccination revealed a specificity of 86.4% DPPD that was much improved over the 42.1% observed for PPD (Badaro et al. 2020).

Leprosy (Hansen's disease)

Manifesting from *Mycobacterium leprae* infection, leprosy (Hansen's disease) is a leading cause of non-traumatic peripheral neuropathy. The extensive provision of curative multiple drug therapy (MDT) over the last 30 years has reduced the prevalence of this complex disease, but a steady detection rate is now being maintained (WHO 2010). Ongoing *M. leprae* transmission is indicated by case detection rates among children below 15 years of age (Sachdeva et al. 2011a; Singal et al. 2011), and the impact of continued transmission on the true incidence of leprosy is continually identified as a priority by WHO (Sachdeva et al. 2011b). As with TB, epidemiological screening tools that enable early detection of *M. leprae*–infected individuals are deemed critical for identifying those individuals most in need of prophylactic intervention.

Leprosy presents across a wide array of symptoms that can complicate diagnosis even by experts (Ridley and Jopling 1966; Scollard 2004). Patients are often in clinics with limited facilities and WHO diagnostic criteria has been simplified to allow widespread adoption of either one of two recommended treatment regimen. Leprosy patients are defined into the operational categories, multibacillary (MB) or paucibacillary (PB). At the extreme MB pole, LL patients demonstrate high titers of anti-*M. leprae* antibodies but an absence of an inflammatory T cell response (Ridley and Jopling 1966). PB patients, encompassing TT and a number of BT forms, are currently characterized as having one or few skin lesions and granulomatous dermatopathology. PB patients have low infectious burdens and low or absent antigen-specific antibody responses, but they demonstrate a specific cell-mediated immunity against *M. leprae* that provides hope for alternate T cell–based diagnostic strategies, including skin tests.

Skin testing for leprosy has occurred with various antigen preparations generated by crude fractionation of *M. leprae* (Lepromin A, Rees Antigen, Dharmendra, and Convit's antigen) over the years. These have proven safe, and lepromin A has been used for nearly half a century (Convit et al. 1992; Dharmendra 2012; Meyers et al. 1975; Millar et al. 1975). Given the inability to culture *M. leprae* in vitro, however, each of these complex mixtures was derived from *M. leprae* grown in animals (or extracted from human lesions). These processes neither allow for consistent mass production nor do they meet modern safety standards (Meyers et al. 1975). "Semi-refined" antigen preparations incorporating over 100 components reflective of those contained within Convit and Rees were generated more recently under good manufacturing practices (GMP). Upon clinical evaluation, although *Mycobacterium leprae* soluble antigens devoid of glycolipids particularly lipoarabinomannan (MLSA-LAM)) and MLCwA (*M. leprae* cell wall associated antigens) were well-tolerated, the advancement of GMP MLSA-LAM and MLCwA could not be justified as sensitivity was only 20–25% (Marques et al. 2008; Rivoire et al. 2014a, b).

Analogous to the situation with TB, we used in vitro antigen recall data to prioritize the recombinant chimeric LID-1 fusion protein as a skin test candidate antigen for leprosy/*M. leprae* infection. LID-1 was formulated to achieve maximum performance at a minimal dose in preclinical evaluation of DTH in *M. leprae*–immune guinea pigs. Select formulations and doses were then evaluated in armadillos, the only *M. leprae* infection model that recapitulates human disease (Truman et al. 2014). Data indicated that intradermal inoculation of formulated LID-1 could satisfactorily distinguish uninfected from *M. leprae*–infected animals manifesting with symptoms distinctly similar to the PB presentation of patients, suggesting that evaluation among various groups in leprosy-endemic regions is merited.

Skin tests in immune-compromised subjects

Among groups for whom immunological-based TB testing results are traditionally poor, Mtb infection is especially problematic in immune-compromised individuals that have an increased likelihood of progression to disease such that TB is a leading cause of death in HIV/AIDS patients. The use of TST for the diagnosis of active disease in HIV-infected individuals has been controversial with previous studies reporting reduced sensitivity

in this population (Goldstein et al. 1994; Graham et al. 1992; Johnson et al. 1992; Lifson et al. 1993; Mamani et al. 2013; Whittle et al. 1993).

CD4 T cell counts are likely a major determinant of the induration size following skin testing in HIV-positive TB patients. Accordingly, although expanded testing among individuals with defined CD4 T cell counts is required to validate it, we observed that the magnitude of response to DPPD correlated strongly with CD4 T cell counts of HIV-infected patients. Whereas sensitivity of the standard TST test in this population is less than 50%, the DPPD-based test gave 90% sensitivity including even in individuals with very low CD4 T cell counts. These data indicate a potentially significant benefit of skin testing over ex vivo assessment of small blood volumes, likely because antigen-specific T cells that may be limited within the small blood volumes used for IGRA are afforded the time to relocate to, and accumulate at, the antigen inoculation site in a skin test. Thus, defined skin tests such as DPPD have both specificity (i.e., non-reactive to BCG or mycobacteria other than Mtb) and sensitivity (i.e., able to detect Mtb in immune-compromised individuals) advantages over traditional TST.

Logistics and economics of skin testing

The use of lab-based IGRA and NAAT in resource-limited settings has been restricted by their cost and modest availability (Morrison et al. 2008; Pai et al. 2014; Rangaka et al. 2012). Even in the USA, 2013 private insurance claims data relating to TB testing indicated that IGRA were used far less often than TST (13.7% versus 86.3%, respectively) (Owusu-Edusei Jr. et al. 2017). This was despite medical expenditures for TB-specific tests among the employer-based privately insured population costing an estimated $53.0 million. TST was not only the most commonly used test but also provides a clear economic advantage as it was, by far, the least expensive ($9) (Owusu-Edusei Jr. et al. 2017). In addition, skin testing provides a practical advantage as it is well-suited for use in decentralized field studies or surveillance programs where even rudimentary laboratory support is lacking (Fig. 1).

Beyond mycobacteria...

As exemplified by PB leprosy, skin tests lend themselves to detection of pathogens that cause low-level infections that do not elicit strong antibody responses but do elicit inflammatory CD4 T cell responses. Accordingly, the majority of people with asymptomatic, self-resolving *Leishmania donovani* and *L. infantum* infections, and cutaneous or mucosal leishmaniasis patients, have positive responses in the leishmanin (Montenegro) skin test commonly produced from cultured promastigotes that are washed and inactivated in 0.5% phenol saline (Magill 2013) or, more recently, with parasite extract (Reed et al. 1986).

Of particular current interest, it is noteworthy that early diagnostic efforts in response to the COVID-19/SARS-CoV-2 pandemic focused on nucleic acid– and antibody-detection tests, but both these platforms have left testing gaps. Antibody-based tests typically fill the void that nucleic acid detection leave (i.e., fleeting positivity and a lack of information relating to history of infection or immune status), but the rapid waning of antibody titers is a natural phenomenon common to coronavirus immunity also been reported after SARS-CoV-2 infection (Alshukairi et al. 2016; Callow et al. 1990; Ibarrondo et al. 2020; Long et al. 2020; Shin et al. 2019). Detailed evaluations are now indicating that antigen-specific T cell responses generated by both symptomatic and asymptomatic SARS-CoV-2 infection are more stable and longer-lasting than humoral responses. Thus, given the inherent benefits documented above, there appears to be significant merits in developing SARS-CoV-2-specific skin tests for integration into COVID-19 epidemiology and containment strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any experiments with animals or human participants performed by any of the authors.

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