PrimaTB STAT-PAK® Assay, a Novel Serodiagnostic Test for Tuberculosis in Nonhuman Primates

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ABSTRACT

Background: Tuberculosis is the most important bacterial disease in nonhuman primates. The current diagnostic method, the tuberculin eyelid test, has considerable shortcomings. We characterized antibody responses against Mycobacterium tuberculosis and M. bovis in macaques to develop a rapid serodiagnostic test for tuberculosis.

Methods: Twenty-seven monkeys were infected: 14 rhesus (Macaca mulatta) and 7 cynomolgus (Macaca fascicularis) inoculated with M. tuberculosis, and 6 rhesus inoculated with M. bovis. Twenty-one additional rhesus monkeys were inoculated with M. tuberculosis post-infection by MultiAntigen Print ImmunoAssay (MAPIA™) using 13 recombinant antigens.

Results: All animals produced antibodies of variable levels and with differential antigen recognition patterns. Three proteins, ESAT-6, CFP10, and MPB83, were most frequently recognized and a combination of these antigens was sufficient for antibody detection in all 27 infected monkeys. The antigen cocktail was optimized and used to develop a rapid serodiagnostic test for tuberculosis, PrimaTB STAT-PAK® Assay, based on lateral-flow technology. By varying the protein starting in weeks post-infection, 26 out of the 27 infected monkeys were read as true in the test. Only 3 false-positive samples were found among 195 sera from control monkeys.

Conclusion: The serodiagnostic assay is an improvement over the current tuberculin eyelid test in identifying infected monkeys. It is a simple and user-friendly assay that is easy to perform and read. It is stable at room temperature for up to 18 months, and requires no laboratory equipment or skilled personnel. The test can use serum, plasma or whole blood samples to provide results within 20 minutes. The PrimaTB STAT-PAK® Assay was compared to MAPIA for sensitivity and specificity.

INTRODUCTION

Tuberculosis is the most important bacterial disease in nonhuman primates because of its ability to spread rapidly, its high fatality rate, and its zoonotic potential. The primary diagnostic method for tuberculosis in monkeys, the tuberculin eyelid test, has considerable shortcomings. We characterized the humoral immune responses in monkeys experimentally infected with Mycobacterium tuberculosis or M. bovis to develop a rapid serodiagnostic test for tuberculosis in nonhuman primates. The results demonstrated animal-to-animal variation of antigen recognition patterns, and that a combination of three selected antigens is required for detection of antibodies in all infected monkeys.

METHODS

Sera. Serum samples were sequentially collected from 27 monkeys: 21 infected with M. tuberculosis and 6 with M. bovis for 4 months after infection (Table 1). A large control group of 195 sera included 6 rhesus macaques experimentally infected with M. avium (n=3) or M. kansasii (n=3).

MAPIA™ (MultiAntigen Print ImmunoAssay) was performed as previously described (Lyashchenko et al. 2000) using a panel of 13 recombinant antigens.

PrimaTB STAT-PAK® Assay. A rapid antibody detection assay was developed by Chembio Diagnostic Systems, Inc. (Figure 2). This test uses the lateral-flow technology and a cocktail of 3 recombinant antigens of M. tuberculosis and M. bovis to detect antibodies to TB in nonhuman primates. PrimaTB STAT-PAK® Assay is a simple and user-friendly assay that is easy to perform and read. It is stable at room temperature for up to 18 months, and requires no laboratory equipment or skilled personnel. The test can use serum, plasma or whole blood samples to provide results within 20 minutes. The PrimaTB STAT-PAK® Assay was compared to MAPIA for sensitivity and specificity.

RESULTS

1. All monkeys produced antibodies during experimental infection. Antigen recognition patterns varied from animal to animal (Figure 1).
2. Two antigens, ESAT-6 and MPB83, were most frequently recognized (Table 2 and 3), and ESAT-6 and M. bovis IFNγ-PST were sufficient for detection of IgG antibodies in all infected monkeys by PrimaTB STAT-PAK® Assay.
3. Twenty four of 27 infected monkeys were positive by PrimaTB STAT-PAK® Assay. Only 3 false-positive samples were found among 195 sera from control monkeys (Table 4). Sensitivity methods showed more consistent results than did the tuberculin eyelid test in identifying infected monkeys (Table 5).

CONCLUSION

PrimaTB STAT-PAK® Assay showed a diagnostic sensitivity of 86.9% and a specificity of 98.5%. This test should provide a rapid and accurate diagnostic tool for the improved control of tuberculosis in nonhuman primates.