Published in final edited form as:

Med Hypotheses. 2012 September ; 79(3): 365–367. doi:10.1016/j.mehy.2012.05.037.

TB infection in the nonhuman primate biomedical model: tip of the iceberg?

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Abstract

Biomedical research in the 21st century increasingly relies on pathogen-free nonhuman primates (NHP) to model human pathophysiology. Despite adherence to protocols designed to maintain pathogen-free colonies, reports of tuberculosis regularly appear. We hypothesize that, undetected by standard screening protocols, mycobacteria of the *Mycobacterium tuberculosis* complex (MTBC) continue to circulate in established NHP colonies and may, in addition, be periodically reintroduced with newly imported animals. The tuberculin skin test (TST), the accepted standard screening test for tuberculosis, relies on the host's immune response to detect infection, but empirical data suggest that TST lacks both specificity and, particularly in certain NHP species and in immune compromised animals, sensitivity. In order to improve the detection of MTBC infection in NHP colonies we propose new screening protocols that incorporate molecular methods to detect mycobacteria. These new tests do not rely on the host's immune response and may allow for strain typing of the pathogens – enhancing our ability to elucidate patterns of disease transmission. Moreover, the ability to rapidly and noninvasively collect specimens could lead to an improved appreciation of the burden of MTBC circulating in populations of NHP and humans, including drug-resistant strains, data that are invaluable to public health efforts.

INTRODUCTION

Over the past decades nonhuman primates (NHP) have been widely incorporated into biomedical research as models of human physiology and pathophysiology. As the closest living relatives to humans, NHP share genetic, immunologic, and behavioral characteristics

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Contributors:

The original idea for this Hypothesis arose from discussions between AK Wilbur and L Jones-Engel. All authors were involved in the writing of the drafts and final submission.

Conflicts of interest:

None

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that make them sought after models for human health research. The NHP model has been a critical resource for advances in a variety of fields, including AIDS, polio, malaria and influenza. By far the most commonly used NHP species are the rhesus (*Macaca mulatta*), pigtailed (*M. nemestrina*) and long-tailed (*M. fascicularis*) macaques. These species, those being bred in captivity in the US as well as those wild-caught and imported from habitat countries in Asia, account for the majority of the 112,000 captive NHP in the US. It is probable that they will, for the foreseeable future, continue to play an important role in a diverse range of biomedical research.¹ To ensure the validity of primates as experimental models, it is vital that, whenever possible, significant pathogens that infect resident and newly-imported macaques be detected.

SPF colonies: a higher standard in experimental models

Preventing, detecting, and treating infectious disease remain major challenges at NHP facilities. Many of these infectious diseases can lead to high morbidity and mortality. Loss of animals to illness is costly. Moreover, given the comparatively small numbers of NHP available for research (compared to, for example, rodents, or fruit flies) each animal is valuable, and the loss of even a few experimental subjects can affect the results of an experiment or set a project back months or even years. Of equal importance from the scientific perspective, disease can potentially cause biological effects that confound experimental results. Thus, it is critical to prevent infection whenever possible, or at least to identify its presence^{2,3}. In order to address the threat of disease, NHP research labs developed specific pathogen-free (SPF) colonies, which were originally defined as colonies devoid of *Mycobacterium tuberculosis* complex (MTBC) bacteria.⁴ The SPF designation has subsequently been modified to exclude several viruses. SPF colonies represent a higher standard in the NHP biomedical model: results of research using SPF animals are thought less likely to be confounded by the effects of infection, and risks to husbandry and research staff are minimized.

The pathogens of greatest concern to NHP laboratories have historically been mycobacteria of the MTBC⁴. NHP share with humans a susceptibility to mycobacterial diseases, and newly imported NHP originate from areas of high human MTBC endemicity, where they may be exposed naturally⁵. Research facilities have adopted rigorous procedures designed to detect and prevent the introduction of infected animals, but tuberculosis outbreaks in established NHP facilities continue to occur, leading to the conclusion that at least some of these outbreaks arose from reactivation of undetected latent infections^{4,6}. It has become increasingly clear that our decades-old protocols designed to prevent the introduction and transmission of MTBC are inadequate; it is also clear that undetected MTBC infection is a potential confounder of great importance, especially in HIV research.

Currently used tests fail to reliably detect MTBC

Arguably, immunocompromised animals are the laboratory NHP in whom detection of MTBC is most critical, as they are potentially the most vulnerable to the pathological effects of MTBC. In humans, MTBC-HIV coinfection rapidly depletes the cellular immune system and disrupts the T-helper1/Helper2 balance⁷; active tuberculosis doubles the risk of death in HIV-infected individuals⁸. Research on MTBC-SHIV (Simian/Human Immunodeficiency Virus is a man-made SIV/HIV chimera) coinfecting NHP indicates that the pathogenic synergy of these two pathogens in NHP is similar to that seen with MTBC-HIV coinfection in humans⁹. Detecting mycobacterial co-infection in SHIV-infected animals is complicated however, by SHIV-related anergy. The standard test for diagnosing MTBC infection in laboratory NHP is the Tuberculin Skin Test (TST), which relies on the host's intact immune function. Laboratory protocols call for the discontinuation of TST in animals experimentally

infected with SHIV, as a negative result is expected even in the presence of mycobacterial infection. Ironically, these animals may be most at risk for severe mycobacterial disease.

It has been argued that MTBC infection inevitably, when it is present in laboratory primates, becomes manifest. For many years MTBC was thought to cause widespread, fulminant disease and rapid mortality in NHP populations. Recent data give a more nuanced picture. In rhesus and longtailed macaques, in which experimental infection with *M. tuberculosis* is best studied, active bacterial replication has been documented in experimentally infected animals, in the absence of clinical disease¹⁰. In experimentally infected rhesus and long tailed macaques, *M. tuberculosis* causes a broad spectrum of disease similar to that seen in humans, from latency to fulminate disease with high mortality¹⁰. Still, little is understood about the factors that influence mycobacterial pathogenicity in NHP, and little to nothing is known about the progression of mycobacterial infection in other NHP species, including the pig-tailed macaques, a favored laboratory model for research on HIV. Thus, with an incomplete understanding of the range of disease caused by mycobacteria in NHP and diagnostic tests that unreliably detect the presence of the pathogen, it is entirely possible that mycobacteria could circulate in and be repeatedly introduced into lab NHP populations, without the knowledge of researchers and laboratory personnel.

Newly available tests may improve the detection of MTBC

Taken together, these observations lead us to hypothesize that MTBC has gone undetected in our NHP imports for decades, resulting in established colonies with undiagnosed MTBC infections that result in the periodic outbreaks reported throughout the literature. Our current screening methods are capable of detecting only a very tiny subset of these infections: the tip of the iceberg. An ideal screening test for MTBC would be highly specific, highly sensitive in detecting infection, including asymptomatic and latent infections, rapidly and safely applied, and easily interpreted. The test would detect the organism before it had been transmitted to other individuals, allowing for effective quarantine and treatment measures.

The current standard for tuberculosis screening in NHP colonies, the TST, is far from the ideal. It has limited sensitivity, repeatedly failing to detect TB infections in the lab context.¹¹ TST may also yield false positive results, which can be observed in animals that are infected with nontuberculous mycobacteria.⁶ The TST is performed in laboratory NHP by injecting purified antigen into a sedated animal's eyelid. The result is then assessed subjectively after 48–72 hours as the amount of resultant eyelid swelling. TST's reliance on subjective evaluation of the host's immune response to antigenic challenge is a significant drawback. Moreover, even in immune competent NHP, there is evidence that immune response to both MTBC infection and TST may differ significantly among NHP species^{12;13}. Given the distinct evolutionary ecologies among NHP species used in research, it is not only unsurprising, but expected, that host and pathogen coevolutionary relationships have evolved very different adaptations between MTBC and NHP species¹⁴. In relying on TST to detect MTBC infection, NHP laboratories are using an insensitive, non-specific test on a broad range of hosts with diverse immunological systems. It is thus not surprising that MTBC periodically emerges in these settings, only that it is reported so infrequently.

Clearly, tests with improved diagnostic accuracy are needed to ensure that SPF colonies truly are pathogen free. Newly available screening tests that rely on detecting the pathogen itself have the potential to improve the sensitivity and specificity of TB detection in laboratory NHP. These tests are already widely used to diagnose TB in humans; molecular methods employing polymerase chain reaction (PCR) have been used for over a decade to detect the presence of MTBC DNA in human bronchoalveolar lavage, gastric aspirate and sputum samples¹⁵. These methods detect DNA sequences (the standard is the IS6110 repetitive fragment) that are unique to MTBC in the organs that are most often affected.

PCR-based tests have also been used to amplify IS6110 from tracheal wash samples from *M. fascicularis*¹⁶. In contrast to TST, these molecular methods do not rely on the intact immune function of a specific host species. Another important advantage of a PCR-based test is its potential to inform genotypic analysis. Phylogenetic analysis, taking advantage of recent whole-genome studies and GenBank's growing collection of various mycobacterial sequences, can determine patterns of MTBC transmission, crucial data that can be used to prevent the transmission of MTBC within a colony.

As MTBC is presumed to affect the NHP respiratory and/or gastrointestinal systems, it is likely that the mouth and nose are routes for the entry and exit of infectious organisms, and are thus a potential anatomical location from which MTBC could be detected. Over the past years we have collected swabs of the oral mucosa for analysis by PCR and our group recently published data indicating that MTBC DNA can be detected by PCR amplification of IS6110 in buccal swabs of free-ranging NHP that live among humans in high tuberculosis prevalence areas¹⁷. Oral Swab PCR (OSP) could combine the advantages of a non-host immune-based test with the advantages of an easily administered and minimally invasive test.

DISCUSSION

Tests such as OSP, which screen directly for the pathogen, provide an opportunity to detect MTBC in a group of exposed/infected animals even in the absence of an observable immune response. There are also other important benefits to including a direct pathogen-based test among our screening procedures. With OSP the ease with which biological samples are acquired will allow the logistically manageable collection of larger numbers of samples and greater sampling frequency, resulting in a more accurate measurement of the MTBC prevalence within a population. In addition, strain-typing will provide a better view of the genotypic range of MTBC as well as patterns of MTBC transmission. Detection and ascertainment of provenience of MTBC in laboratory NHP might prove a crucial variable; if the origin of the MTB infection is a co-evolved (indigenous) NHP strain, virulence may be minimal. Exogenous transmission, on the other hand, especially from humans, might be expected to be more pathogenic, resulting in more severe disease.¹⁸ Currently employed diagnostic tests often fail to detect subclinical tuberculosis, and cannot determine the provenance of the pathogen.

Development of detection and ecotyping tools such as the OSP can improve our characterization of the pathogen landscape, setting the stage for advances in the study, treatment and prevention of tuberculosis. Our knowledge of the MTBC landscape has heretofore been limited to the subset of strains in exposed individuals who developed clinical disease. Better elucidation of the pathogen landscape would be a powerful tool for research on the factors that influence the transmission of tuberculosis as well as the properties of the pathogens themselves that influence host response to MTBC. Progress in these areas could, in turn, have important ramifications for the development of antitubercular antibiotics and vaccines.

Acknowledgments

The authors are grateful to Dr. Nicholas Lerche who strongly believed that an interdisciplinary approach to infectious disease research was critical for a complete and nuanced understanding of our biomedical models and research outcomes. This research was supported by funding from NIH-NCRR grant P51 RR000166 and RR 02S014, NIH-NIAID grants R01 AI078229 and R03 AI064865, N66001-02-C-8072 DARPA, Unconventional Concepts Inc.

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