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ScienceDirect
Microbes and Infection 8 (2006) 2587–2598
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

High resolution radiographic and fine immunologic definition of TB disease progression in the rhesus macaque

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Received 20 July 2005; accepted 6 July 2006
Available online 14 August 2006

Abstract

Mycobacterium tuberculosis infection in non-human primates parallels human tuberculosis, and provides a valuable vaccine evaluation model. However, this model is limited by the availability of real-time, non-invasive information regarding disease progression. Consequently, we have combined computed tomography scanning with enumeration of antigen-specific T cell responses. Four rhesus monkeys were infected with *M. tuberculosis* strain H37Rv (1000 cfu) in the right lower lobe via a bronchoscope. All uniformly developed progressive tuberculosis, and required euthanasia at 12 weeks. Computed tomography scanning provided detailed real-time imaging of disease progression. At necropsy, computed tomography and pathohistologic findings were tightly correlated, and characteristic of human disease. Immunologic monitoring demonstrated progressive evolution of high frequency *M. tuberculosis*-specific $CD4^+$ and $CD8^+$ T cell responses. Peripheral blood effector cell frequencies were similar to those observed in tissues. In summary, computed tomography scanning in conjunction with immunologic monitoring provides a non-invasive, accurate, and rapid assessment of tuberculosis in the non-human primate.
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Keywords: Tuberculosis; CT scanning; Macaque

1. Introduction

Tuberculosis (TB) remains a leading cause of infectious disease mortality worldwide, accounting for nearly two million deaths annually [1]. TB eradication will require the development of an improved vaccine. The development of a vaccine will, in turn, depend upon an improved understanding of the mechanisms underlying protective immunity, and the ability to design and test candidate vaccines in the context of a model that will be predictive of efficacy in humans.

Abbreviations: APC, antigen presenting cells; BAL, bronchoalveolar lavage; BCG, Bacille Calmette–Guérin; CT, computed tomography; CXR, chest radiograph; ELISPOT, enzyme-linked immunospot assay; ESR, erythrocyte sedimentation rate; IFN-γ, interferon gamma; LCL, lymphoblastoid cell lines; MOT, Mammalian Old Tuberculin; TB, tuberculosis.

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1286-4579/$ - see front matter Published by Elsevier Masson SAS.
doi:10.1016/j.micinf.2006.07.007

Mice, guinea pigs and rabbits have been employed in pre-clinical evaluation of TB vaccines. Each model system demonstrates distinctive advantages and disadvantages and each will likely contribute to demonstration of vaccine safety and efficacy [2]. However, TB in each of these animals is clinically and immunologically distinct from human TB and no current vaccine candidate performs substantially better than the Bacille Calmette–Guérin (BCG) vaccine in these models. Thus evaluation in a non-human primate model will likely be required before large-scale human evaluation can occur. Indeed, the Aeras Global TB Vaccine Foundation has included evaluation of the non-human primate model as a critical component of pre-clinical vaccine evaluation (http://aeras.org).

Mycobacterium tuberculosis (Mtb) infection in Old World monkeys has many parallels with human TB. The infection can be naturally spread via aerosol contact, conditions such as over-crowding and poor nutrition dramatically increase the likelihood of disease spread and progression [3], and the most common manifestations of disease are similar to humans including pulmonary granulomata with calcification, caseation, cavitation; and infection of draining tracheobronchial lymph nodes. Furthermore, extrapulmonary manifestations of disease have been reported. Finally, presentation of clinical TB after rigorous tuberculin screening has been reported, suggesting that chronic persistent (latent) infection may occur [4,5].

Experimentally, Mtb infection has been studied in both the rhesus and cynomologus monkeys. Following aerosol infection, rhesus macaques develop human-like extensive pulmonary disease followed by hematogenous spread to the liver, spleen, and kidney [6,7]. Following intratracheal infection, cynomologus monkeys develop similar pulmonary disease [8]. Evidence that cynomologus monkeys challenged with low-dose intrapulmonary Mtb can develop latent infection [9] and the ability to perform detailed immunological analysis in monkeys further strengthens the case for using non-human primate models to develop and refine TB vaccination strategies. Finally, rhesus monkeys may demonstrate a potential advantage over cynomologus monkeys because the latter may be more resistant to Mtb infection [8,10], and are better protected following immunization with subcutaneous BCG [10]. Similarly, the failure of BCG to protect in the rhesus model may suggest that the rhesus monkey model may be able to delineate vaccine candidates superior to BCG [10].

To date, non-invasive monitoring of disease via clinical (weight loss and fever) and laboratory (C reactive protein, erythrocyte sedimentation rate [ESR], and chest radiograph [CXR]) indicators have had limited utility in the evaluation of disease progression. As a result, evaluations of vaccine efficacy have relied upon post-mortem pathological analysis and/or time to death as primary endpoints. The absence of non-invasive tools to define vaccine efficacy represents a critical limitation of the non-human primate model.

The aim of this study was to establish the ability of computed tomography (CT) scanning and immunologic monitoring to accurately measure the extent and character of infection with Mtb. The ultimate goal is to employ these markers as accurate surrogates for disease burden to improve TB rhesus monkey vaccine model.

2. Materials and methods

2.1. Experimental animals

Four specific pathogen free (Simian Immunodeficiency Virus, Cercopithecine Herpesvirus 1, Type D Simian Retrovirus Serotypes 1, 2, 5, and Simian T-lymphotrophic Virus 1) male juvenile (30 to 32-month-old) rhesus monkeys (*Macacca mulata*) were enrolled (Oregon Regional Primate Research Center, Beaverton, OR, USA). Animals were placed in animal biosafety level 3 containment four weeks prior to the initiation of the protocol. Protocols were approved by the Institutional Animal Care and Utilization Committee.

2.2. Mtb strains and culture

Mtb strain H37Rv expressing luciferase was kindly provided by Dr. Douglas Young and stocks were cultured as previously described [11]. To perform quantitative cultures, serial dilutions of bronchoalveolar lavage (BAL) fluid or organ homogenates were plated in duplicate.

2.3. Bronchoscopy and instillation of Mtb

Macaques were sedated with ketamine (10–20 mg/kg, IM) and atropine (0.05 mg/kg, IM). Bronchoscopy was performed by a pulmonologist (SA or DML). Just prior to inoculation, the Mtb stock was thawed and sonicated for four 30-s bursts in a Sonic Dismembrator MDL60 (Fisher Scientific). Mtb (1000 cfu) was mixed thoroughly in saline (3 mL total volume), and instilled into the right lower lobe of each subject via bronchoscope (4.5 mm external diameter; Olympus). For bronchoscopic lavage, the bronchoscope was wedged into the right lower lobe. Three 10-mL instillations were performed, with an average return of 20 mL. Following instillation of Mtb, subjects were kept with the right side dependent for at least 5 min.

2.4. Clinical monitoring

Subjects were sedated monthly for the determination of temperature, weight, blood draw, and intradermal placement of either palpebral or abdominal Mammalian Old Tuberculin (MOT; Symbiotics). Subjects with a prior positive MOT were not re-tested. Bronchoscopy was performed 5 days following CT scanning to avoid visualizing residual lavage fluid. The time of necropsy was determined by the veterinary staff, following guidelines described below. Determinations of complete blood count and ESR were performed by the Oregon National Primate Research Center Clinical Laboratory.