Microbial Pathogenesis 50 (2011) 39–47

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Comparison of rhesus and cynomolgus macaques in a *Streptococcus pyogenes* infection model for vaccine evaluation

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ARTICLE INFO

Article history:
Received 1 September 2010
Received in revised form
20 October 2010
Accepted 21 October 2010
Available online 28 October 2010

Keywords:
Streptococcus pyogenes
Monkey infection

ABSTRACT

Animal models predictive of human disease are generally difficult to establish and reproduce. In the case of the Group A Streptococcus (GAS) bacterium, which is predominantly a human pathogen, virulence assessment in animal models is problematic. We compared a monkey colonization and pharyngitis model of infection in two macaque species to determine the optimal model for vaccine candidate evaluation. Rhesus and cynomolgus macaques were intranasally infected with a streptomycin resistant (Str^r) GAS strain. Monkeys were monitored for body weight and temperature changes, throat swabs and sera were collected, and clinical observations were noted throughout the study. Both species exhibited oropharyngeal colonization by GAS, with rhesus macaques demonstrating a more sustained colonization through day 28 post-challenge. Veterinary observations revealed no significant differences between GAS-infected rhesus and cynomolgus macaques. Mock-infected monkeys did not exhibit clinical symptoms or GAS colonization throughout the study. ELISA results demonstrated that both rhesus and cynomolgus macaques developed anti-streptolysin-O antibody titers, with cynomolgus generating higher titers. Sera from infected monkeys produced opsonophagocytic killing and bound to the bacterium in an immunofluorescence assay. Both rhesus and cynomolgus macaques can be used for colonization studies with this GAS M3 strain, yet only mild clinical signs of pharyngitis and tonsillitis were observed.

1. Introduction

The bacterial pathogen *Streptococcus pyogenes* (Group A Streptococcus, GAS) is a human pathogen. *S. pyogenes* is responsible for a variety of diseases, including pharyngitis, cellulitis, impetigo, necrotizing fasciitis, scarlet fever, septicemia, toxic shock syndrome, and the nonsuppurative sequelae, acute rheumatic fever and acute glomerulonephritis. Morbidity and mortality of GAS associated diseases is continually increasing. Worldwide, Group A Streptococcus accounts for over 616 million cases of pharyngitis each year [1]. Due to the overwhelming burden of GAS disease, a vaccine is desperately needed.

Reproducible animal models for this bacterium and its associated diseases are lacking, because they do not adequately replicate human pathogenesis and in most cases no immune sequelae result. The lack of animal models poses a major challenge for GAS vaccine development. As early as the late 1800s, scientists have attempted to reproduce a rheumatic fever model in rabbits [2,3]. Since that time, Group A Streptococcal infection models have been developed in zebrafish, mice, rats, rabbits, pigs and non-human primates to mimic associated diseases [4–10] (not inclusive). Non-human primate models of GAS infection have shown promise because the monkeys develop pharyngitis following inoculation with the bacterium, supporting the clinical relevance of the model. Monkeys evaluated in the aforementioned study developed an increase in tonsil size and pharyngitis after intranasal infection with GAS [11]. Rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), baboons (*Papio spp.*) and chimpanzees (*Pan troglodytes*) have been used in GAS immunogenicity and/or infection studies [10–20]. In an effort to further characterize vaccine candidates that demonstrated protection from GAS intranasal challenge in mice, we developed a GAS intranasal monkey challenge model. Here we describe a comparison of GAS intranasal infection in rhesus and cynomolgus macaques, and the associated immune and biological responses to these infections.

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0882-4010/$ – see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.micpath.2010.10.004

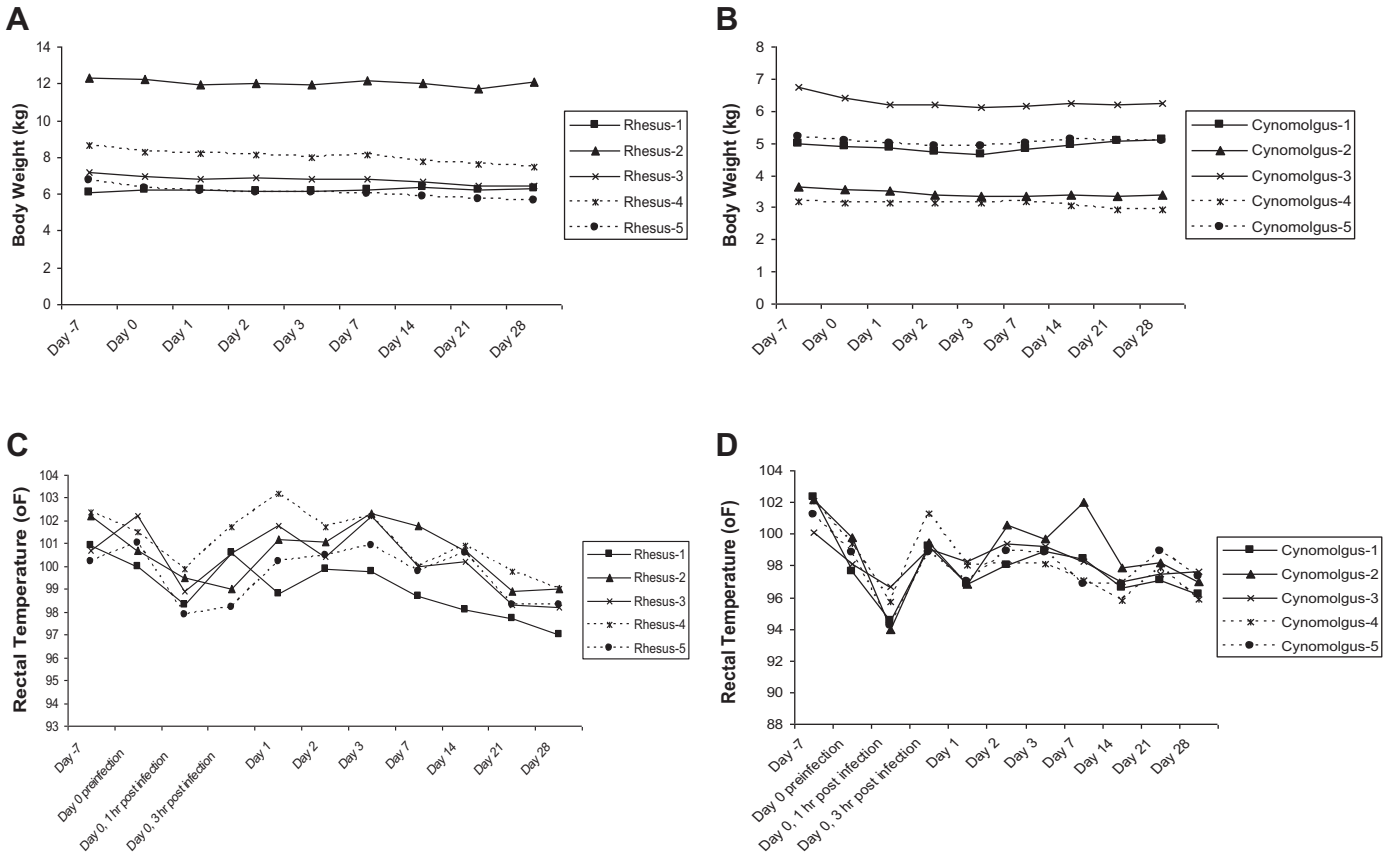

Fig. 1. Rhesus and cynomolgus monkey monitoring over the course of the study for GAS infected and PBS controls. Rhesus (A, C) and cynomolgus (B, D) body weights and temperatures, respectively. Solid lines represent monkeys infected with Str^r GAS M3; dashed lines indicate mock infected monkeys.

2. Results

2.1. Infection of rhesus and cynomolgus macaques with streptomycin resistant (Str^r)GAS M3

Prior to the study start (Day −7), all monkeys were confirmed to be free of colonization by GAS in the oropharynx. On Day 0, each monkey enrolled in the study was intranasally delivered 1 ml of either Str^r GAS M3 or phosphate buffered saline (PBS) as a negative control. At each time point, temperature and body weight were monitored for each monkey (Fig. 1). Both rhesus and cynomolgus macaques did not demonstrate any fluctuation in weight (Fig. 1A and B) and temperatures were within the normal range, as determined by the veterinarian. The drop in temperature observed on Day 0, (1 hour post-infection) was attributed to the effect of sedation at this early time point (Fig. 1C and D).

Previously, investigators have reported that monkeys infected with GAS developed clinical signs of infection that were consistent with those observed in humans naturally infected with this organism. Virtaneva et al. reported that cynomolgus macaques developed an increase in tonsil size and pharyngitis score after infection with GAS strain MGAS5005, a serotype M1 strain [11]. In order to directly compare our results with those previously reported, the monkeys in this study were scored for tonsil size and pharyngitis based on the same criteria [21]. The monkeys were monitored throughout the study and a single veterinarian completed all of the scoring. Neither rhesus nor cynomolgus monkeys infected with Str^r GAS M3 exhibited clinical symptoms, tonsillitis or pharyngitis scores above 1+ (mild erythema with hyperemic blood vessels, approximate 25% increase in tonsil size, 0–25% occlusion of oropharyngeal space) (Table 1). Control monkeys, mock infected with PBS, did not develop any clinical signs and remained at a pharyngitis and tonsillitis score of 0 throughout the study.

All mock challenged monkeys remained uninfected throughout the study. Swabs obtained on Days −7 and 0 (pre-infection) did not contain any beta-hemolytic GAS colonies. All infected monkeys had GAS recoverable from the oropharynx beginning shortly after inoculation, as evidenced in the swabs collected at 1 hour post infection. All rhesus, and two of three cynomolgus monkeys remained colonized throughout the course of the study (Day 28). Colonies, selected randomly from each throat swab plate, were evaluated in a PCR assay at each time point. In each case, the amplified product confirmed that the isolated organisms were GAS strains of the correct serotype (M3), while the mock-challenged animals did not produce any DNA fragments of the corresponding size in the colony PCR assay, as expected (data not shown). Cynomolgus-1 did not yield any PCR positive colonies on either Day 21 or 28, thereby confirming that this animal was no longer colonized at this time point (Fig. 2). Cynomolgus-3 also did not yield a positive PCR result in this assay on either Day 1 or 21. While the Day 21 data was anticipated since this animal did not have recoverable GAS at this time point, the lack of an appropriate PCR product on Day 1 was surprising as this animal was colonized by GAS at this time point. This lack of concordance may be due to the sensitivity of the PCR assay or the possibility that the animal was colonized with another streptomycin resistant beta-hemolytic bacterium. All PCR results correlated with GAS monkey colonization, with the exception of the one aforementioned sample.