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Lower respiratory tract infection in cynomolgus macaques (*Macaca fascicularis*) infected with group A *Streptococcus*

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ABSTRACT

Group A *Streptococcus* (GAS), a human-specific pathogen, is best known for causing pharyngitis ("strep-throat") and necrotizing fasciitis ("flesh-eating disease"). However, the organism is also an uncommon but important cause of community-acquired bronchopneumonia, an infection with an exceptionally high mortality rate. Inasmuch as little is known about the molecular pathogenesis of GAS lower respiratory tract infection, we sought to develop a relevant human infection model. Nine cynomolgus macaques were infected by intra-bronchial instillation of either sterile saline or GAS (10^5 or 10^7 CFU). Animals were continuously monitored and sacrificed at five days post-inoculation. Serial bronchial alveolar lavage specimens and tissues collected at necropsy were used for histologic and immunohistochemical examination, quantitative microbial culture, lung and blood biomarker analysis, and in vivo GAS gene expression studies. The lower respiratory tract disease observed in cynomolgus macaques mimicked the clinical and pathological features of severe GAS bronchopneumonia in humans. This new monkey model will be useful for testing hypotheses bearing on the molecular pathogenesis of GAS in the lower respiratory tract.

1. Introduction

Group A *Streptococcus* (GAS) is a significant human pathogen that causes a myriad of infections ranging in severity from innocuous pharyngitis ("strep-throat") to life-threatening necrotizing fasciitis ("flesh-eating disease") [1]. GAS is also an uncommon, but important, cause of community-acquired bronchopneumonia [2,3]. With the worldwide resurgence of invasive GAS disease observed during the past 15–25 years [4], epidemiological studies have reported a concomitant increase in the frequency of GAS bronchopneumonia [5,6]. The lower respiratory tract now accounts for approximately 12% of all invasive GAS infections [3,7–10]. Most patients present sporadically [5,6]; however, large outbreaks of GAS bronchopneumonia have occurred among previously healthy young adult populations such as military recruits [11–14], and smaller outbreaks have been reported within families and among patients in chronic care facilities [15,16]. Notably, the incidence of GAS lower respiratory tract infection is highest among the very young and very old, and the case fatality rate increases progressively with age [6,17]. Mortality ranges from 30 to 60%, a rate much greater than that of necrotizing fasciitis, with fatal outcomes being strongly associated with bacteremia, leukopenia and intensive care unit admission [2,8,17–19]. Severe infections are most common in patients with chronic predisposing conditions such as asthma, bronchiectasis, collagen vascular disease, cystic fibrosis, pulmonary tuberculosis, diabetes, cirrhosis, cancer and substance abuse [3,6,17].

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Inasmuch as GAS bronchopneumonia is a devastating disease with high rates of mortality and very little is known about GAS molecular pathogenesis in the lower respiratory tract, we sought to develop a human relevant animal model. GAS is a host-specialist pathogen that causes natural disease only in humans, and several of its proven and putative virulence factors have modest or no activity against mouse molecules [20,21]. Thus, we have previously used cynomolgus macaques, an animal closely related to humans, as a model organism to study GAS pharyngitis and necrotizing fasciitis [22–24]. Our recent studies with methicillin-resistant *Staphylococcus aureus* (MRSA) also indicated that after lower respiratory tract inoculation, cynomolgus macaques developed MRSA pneumonia that was indistinguishable from human disease [25]. Therefore, we used a similar pulmonary infection model to evaluate GAS virulence in the lower respiratory tract.

2. Results

2.1. Lower respiratory tract infection of cynomolgus macaques

Nine adult male cynomolgus macaques were inoculated via bronchoscopic instillation into the right middle lobe of the lung with either a sham dose (sterile PBS only), low dose (10^5 CFU) or high dose (10^7 CFU) of serotype M3 strain MGAS315 (n = three animals per strain treatment group). Strain MGAS315 was selected because it is genetically representative of epidemic serotype M3 GAS strains causing serious invasive infections in humans, including bronchopneumonia [24,26,27]. The nine animals were assessed for disease progression as described below, with detailed physical examination and specimen collection conducted at 72-h and 120-h post-inoculation. Necropsies were performed following the second examination. Multiple clinical, pathological, microbiological and molecular assays were used to assess the severity of the resulting lower respiratory tract infection, including BAL fluid analysis and fine structure histopathology.

2.2. Clinical assessment of GAS-infected monkeys

Personnel performing clinical assessments were blinded to the strain treatment (PBS sham, low dose GAS, or high dose GAS) used to inoculate each animal. All six GAS-infected monkeys developed a significant lower respiratory tract infection, whereas the three sham-infected monkeys remained unchanged from their baseline level of health (Table 1). Despite being afebrile throughout and showing no signs of toxic shock during the five-day post-inoculation observation period, the six GAS-infected animals demonstrated other non-specific signs of infection such as decreased food intake, increased daytime somnolence, and decreased vigor with flat affect. They also demonstrated transiently pale mucous membranes and an infrequent non-productive cough. Importantly, the 72-h midpoint and 120-h peri-mortem examinations revealed markedly altered pulmonary function and positive lung pathology in all six GAS-infected monkeys (Table 1). Compared to the healthy sham-inoculated control animals, the GAS-infected animals had coarse breath sounds and basilar wheezes, more prominent over the right middle (inoculation site) and lower lung lobe during expiration, consistent with airway obstruction (Table 1). Many also had decreased hemoglobin oxygen saturation as measured by pulse oximetry, indicating a defect in alveolar gas exchange (Table 1). Although the number of GAS-infected animals demonstrating each clinical parameter (lung auscultation and pulse oximetry) of lower respiratory tract infection did not appreciably change from the 72-h to the 120-h examination, these findings were modestly more frequent in the high dose treatment group. Furthermore, compared to their pre-inoculation bronchoscopy examination, all six GAS-infected monkeys developed prominent tracheal constriction, bronchial erythema, and superficial bronchial erosions with purulent lesions (Table 1 and Fig. 1). The abundance and severity of these intra-bronchial lesions as observed by bronchoscopy within each animal modestly progressed from the 72-h to the 120-h examination, particularly in the high dose (10^7 CFU) treatment group (Table 1). In summary, monkeys receiving the higher dose of GAS generally demonstrated more severe clinical and intra-pulmonary disease than those receiving the sham inoculation (Table 1 and Fig. 1). These clinical features mimic those observed in humans with moderate to severe GAS bronchopneumonia [28,29].

2.3. Gross pathology of the infected monkey lungs

To begin assessing GAS virulence in the lower respiratory tract, we conducted visual inspection and manual examination of the lungs excised at necropsy. Lungs from all six GAS-infected monkeys had strong evidence of lower respiratory tract infection, including focal to diffuse pleural discoloration, hemorrhagic parenchyma, and focal to diffuse tissue consolidation (Fig. 2A–C). Although the right middle (inoculated site) and lower lobes were most markedly affected, bilateral changes were present in 3/6 GAS-infected lungs. Five also had increased mass due to accumulation of fibrinopurulent exudate and edema fluid (see microscopy results below). In comparison, lungs taken from the three PBS sham-infected monkeys had only very minor tissue alterations (Fig. 2), probably secondary to trauma introduced by the peri-mortem bronchoscopy procedure. No suppurative pleural effusions, empyemas, overt necrotizing lesions or cavitations were seen.

Table 1
Clinical-pathological data of GAS-infected cynomolgus macaques.

Strain Treatment Group	Positive Lung Auscultation		Decreased Pulse Oximetry		Bronchial Erythemia Present		Bronchial Erosions Present		Purulent Lesions Present		Bronchial Constriction Present	
	72 h	120 h	72 h	120 h	72 h	120 h	72 h	120 h	72 h	120 h	72 h	120 h
PBS	0/3	0/3	0/3 (100,100,99)	0/3 (99,99,99)	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
10^5 CFU	2/3	3/3	1/3 (99,92,99)	2/3 (90,96,90)	3/3	3/3	1/3	1/3	2/3	1/3	0/3	0/3
10^7 CFU	3/3	3/3	3/3 (90,95,90)	2/3 (97,90,92)	3/3	3/3	0/3	2/3	2/3	2/3	2/3	1/3

Animals underwent clinical examination and bronchoscopy at 72-h and 120-h post-inoculation. Numbers shown at each time point refer to the fraction of monkeys in each strain treatment group demonstrating the clinical-pathological feature. Significant alterations were defined as: markedly course breath sounds or marked wheezing being heard by lung auscultation; oxygen saturation being measured at less than 96% by pulse oximetry (actual oxygen saturation values shown in parentheses, with the baseline value for each animal equaling 98–100% at the time of inoculation); and each bronchial feature being observed in multiple locations by bronchoscopy.