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Reemergence of the Murine Bacterial Pathogen *Chlamydia muridarum* in Laboratory Mouse Colonies

Chlamydia muridarum (Cm), the only natural Chlamydial pathogen of mice, has been used extensively to model sexually transmitted *C. trachomatis* of humans[1,2]. In contrast to the genitourinary pathology induced experimentally in translational models, the sites of colonization following natural infection appear to include the gastrointestinal tract and lung[1,3,4]. Colonization results in a robust innate and adaptive immune response in immunocompetent mouse strains and is the cause of the associated pathology[1,2,5]. Described further below, natural infection with Cm has not been reported in laboratory mouse populations since the 1940's, and as such is not routinely screened for by research institutions or commercial vendors. We have recently detected the bacterium in several colonies housed in RARC's vivaria and in incoming shipments of mice from several large academic biomedical research centers. As a result, we have developed a PCR assay in conjunction with a large commercial laboratory animal diagnostic laboratory and have recently implemented testing of all mice imported from other research centers and begun to test existing colonies. Furthermore, we have begun to investigate the prevalence, biology, and pathology of natural infection. A summary of the history and known Cm biology/pathology is provided below.

Cm was first described in the late 1930's and early 1940's when scientists identified a putative infectious organism causing respiratory disease and lung pathology in mice used in studies of influenza and the common cold[13,14]. The causative agent was initially referred to as "mouse pneumonitis virus (MoPn)[15];" it was subsequently identified as a biovar of *C. trachomatis*[6], and finally as Chlamydia muridarum after whole organism sequencing[1,16]. During the initial studies with MoPn, there were two strains of the organism isolated – the Nigg and the Weiss/Chicago strains[1]. Importantly, Cm has not been isolated from laboratory mice since its original discovery. While Cm was likely prevalent in early 19th C laboratory mouse colonies, the development and introduction of modern biosecurity practices, i.e., Caesarean rederivation, in the middle of the 20th C would have been expected to eliminate the organism from most vendors' colonies. Accordingly, routine testing of commercial and research colonies was not and is still not performed. It is unclear how prevalent the organism is in modern laboratory colonies.

Cm is an obligate intracellular bacterium. Like other chlamydia, Cm has two life forms, the non-replicating but infectious "elementary body," and the replicating but non-infectious "reticulate body." Transmission is thought to occur via the fecal-oral route, with lung pathology occurring through aspiration of organisms into the respiratory tract and establishment of low-level infection[1,3]. In experimentally inoculated mice, elementary bodies are observable immediately and reticulate bodies after 5-7 hours[2]. The developmental cycle is approximately 36 hours[1,2]. Gross lesions are detectable ~3 days after inoculation, while cellular response may be detected as early as 90 minutes. There has been no documentation of aerosol transmission[3]. Oral infection has previously been shown to establish long-term, persistent infection in the gastrointestinal

tract, with some studies demonstrating persistence of up to 260 days[3]. The cecum and large intestine are presumptively colonized by Cm after infection, however, there does not appear to be pathology associated with infection. This is similar to Chlamydial infections in other animals, where the gastrointestinal tract is often the natural site of colonization with fecal-oral transmission [3].

In immunocompetent mice, infection typically decreases to a subclinical "steady-state" approximately 20 days following experimental infection[3,17]. IgM and IgG antibodies are detected 7 and 14 days after infection, respectively [1,2,17], and T-cell proliferation returns to baseline levels around 35 days[3]. While initial studies demonstrated apparent resolution of infection, recent studies suggest chronic, subclinical infection in which animals with normal/baseline immunologic parameters remained positive for chlamydial organisms on culture and/or histopathology 50-265 days post-infection[3,6,9]. Nude and other immunodeficient strains appear unable to adequately control infection, with many dying of pneumonia by 30 days[1,7]. Studies have demonstrated that the BALB/c strain has higher Cm burdens, longer infections and greater respiratory pathology as compared to the C57Bl/6 strain[3]. Antibodies and cell-mediated responses develop after initial exposure, however reinfection may still occur[1]. These findings suggest a significant strain difference in Chlamydial infection and host response, with immunocompromised mice appearing more susceptible to colonization and disease[7].

The pathology of Cm infection is primarily the result of the host's innate and adaptive immune responses, rather than a result of bacterial toxins/cytotoxicity[1,2,5]. The acute response is mediated through inflammatory cytokines and neutrophils[1,5]. The adaptive response in mice is mediated by CD4+ T cells and macrophages, with more intense acute responses noted on reinfection[1]. With pulmonary infections, inflammatory infiltrates consisting of CD4+ T lymphocytes, monocytes, macrophages, and heterophils are found in the alveolae. Bronchiolar inflammation may accompany more severe responses[1]. Inflammation persists for a significantly longer duration and leads to more clinically significant issues including alveolar edema in naïve mice[1]. Clinical signs may include dyspnea and tachypnea, lethargy, ill thrift, and weight loss[3]. While colonization and shedding may be prolonged, especially in GI tissues and feces, overt inflammation of the GI tract is not associated with chronic infection[8]. There has been at least one study implicating Cm infection resulting in cardiovascular pathology, including myocardial inflammation and fibrosis in immunocompetent strains, and endocarditis with aortic valvular vegetation, organismal growth in cardiac tissues, and pericardial inflammation in immunocompromised strains[9].

Treatment may be accomplished with antibiotic therapy. Cm appears sensitive to the tetracyclines, including doxycycline[10]. While adverse effects of antibiotic therapy are generally rare and mild, they may be more prevalent and serious in immunocompromised strains[11,12]. Adverse effects include development of gastrointestinal dysbiosis, leading to diarrhea and death. If observed, veterinary assessment should be requested.

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