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A Review of the Cilia-Associated Respiratory Bacillus

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The cilia-associated respiratory (CAR) bacillus is a poorly characterized respiratory tract inhabitant of rodents and rabbits (1-9). The acronym "CAR" was coined by J. Ganaway for use until the bacterium is taxonomically classified (3).

In the first published report on CAR bacillus, a group of aging rats developed clinicopathologic signs resembling chronic respiratory disease, with failure to thrive and dyspnea. Histology revealed bronchocentric lesions including lymphoid hyperplasia, ectasia of the major airways, and mucopurulent exudation (1). Bronchial epithelial hyperplasia and hypertrophy with basophilic striations of the cilia were also seen. The striations were composed of large numbers of filamentous bacteria among and parallel to the cilia. The bacteria were found at all levels of the respiratory tract, including the nasal cavity and middle ears. No causal relationship between the presence of CAR bacillus and the chronic respiratory lesions could be established due to concurrent infections with Sendai virus and perhaps pneumonia virus of mice (PVM) and *Mycoplasma pulmonis*.

In subsequent reports, an organism morphologically similar to CAR bacillus was found associated with pulmonary lesions resembling respiratory mycoplasmosis in wild rats, mice, *Mystromys albicaudatus* and rabbits in the United States, Sweden and Japan (2-9). In most cases, other respiratory pathogens, including *Mycoplasma*, were also present; thus the CAR bacillus was considered an opportunistic invader and not a primary respiratory pathogen (9). However, lesions consistent with chronic respiratory mycoplasmosis in which the CAR bacillus was identified have been recognized in rats free of *Mycoplasma* and viral diseases (4, 5). Similar lung lesions occurred in virus- and *Mycoplasma* antibody-free rats 8 weeks after inoculation with a pure *in vitro* culture of CAR bacillus, suggesting that CAR bacillus can colonize the respiratory tract and induce lesions without the assistance of other pathogens (3). To date, its pathogenicity in laboratory animals is not convincingly established, but its ability to colonize the entire respiratory tract and perhaps contribute to lesion formation raises concern about its presence in animals used in research.

Epizootiology

Information on the epizootiology of the CAR bacillus in research colonies is currently being collected. A previous inability to cultivate this bacterium *in vitro* hampered serologic test development and limited the definitive diagnostic criteria to observation of the organism in silver-stained tissue sections. In 1985, J. Ganaway et al. reported its successful propagation in the allantois of embryonated chicken eggs. This allowed development of an enzyme-linked immunosorbent assay (ELISA) and an indirect fluorescent antibody (IFA) assay for antibodies to CAR bacillus (3). Mammalian tissue culture systems have now been established and are used to produce antigen for immunodiagnostic tests (11). Very little information has been published on the prevalence of CAR bacillus infection or antibodies in laboratory animals. Some data suggest, however, that infection may be common in conventional colonies of rats and mice (11, 19). Conventionally raised rabbits appear to have a more than 90 percent prevalence of antibodies to CAR bacillus (7, 8, 11).

The Organism

Difficulties in cultivating CAR bacillus have delayed its classification. Standard phenotypic criteria, such as biochemical metabolism and growth at various temperatures and pH ranges, cannot be determined as CAR bacillus still cannot be grown in large numbers on cell-free medium. Its characterization has thus far been performed on a rat isolate grown in embryonated eggs (3). The bacillus typically measures 0.2 microns wide

by 6-8 microns long with a triple-layer cell wall and bulbous ends (Figure 1). It is Gram-negative, non-acid-fast, negative periodic acid Schiff (PAS), and non-spore-forming. It is motile but lacks structures resembling flagella, pili or axial filaments, and is tentatively classified with the family of "gliding bacteria" (3). Its possible strain variations, the species-specificity of the different strains, and elaboration of toxins are yet to be explored.



Figure 1. Electron micrograph of CAR bacilli shows elongate bacteria with a triple-layer cell wall.

Clinical Disease and Pathology

Most reports of CAR bacillus in naturally occurring respiratory disease have described animals that were also infected with respiratory pathogens such as *Mycoplasma*, PVM, or Sendai virus (1, 2, 9, 19). However, disease due to naturally occurring, uncomplicated CAR bacillus infection has been reported (4, 5, 16). Unfortunately, the animals in these reports were not tested for antibodies to PVM, sialodacryoadenitis, or rat corona virus. (The complement fixation test used for mouse hepatitis virus in these reports will react with other Corona viruses, but has a low sensitivity.) The extent to which CAR bacillus can contribute to lesion formation in the presence of these recognized respiratory pathogens is unknown.

Clinicopathologic features of naturally occurring, uncomplicated CAR bacillus infection (4) resemble those described in infection and transmission studies (3, 5, 12). Infected rodents are usually asymptomatic (3, 13, 20). Clinical signs, if present, may include weight-loss, rough hair coat, wheezing and rales. Disease-free rats housed with CAR bacillus-inoculated rats are generally asymptomatic, although in one report, rales were detected upon close examination (5). Histologically, the organism can be found colonizing the respiratory epithelium of one- to two-week-old rats born to CAR bacillus-infected dams (3). Histologic lesions in the trachea and lungs consist of mild epithelial hyperplasia of ciliated airway mucosa, with numerous bacilli among the cilia demonstrated by Warthin-Starry (Figure 2), Grocott methenamine silver or immunoperoxidase stains (3, 5). Airways may also contain mucopurulent exudate associated with areas of epithelial necrosis. Lymphocyte infiltration may form hyperplastic lymphoid cuffs or nodules (Figure 3). Mucus, often containing neutrophils and free CAR bacilli, occasionally fills bronchial lumina (Figure 4). Varying degrees of bronchial and bronchiolar ectasia with bronchial abscessation, emphysema, and consolidation of dependent alveoli may also be present (12). Interestingly, clinical signs of respiratory disease have not been observed in naturally infected rabbits (68) or in experimentally infected guinea pigs (14). Histopathologic examination of the respiratory tree in naturally infected rabbits found mild hyperplasia of lymphoid nodules subjacent to the respiratory mucosa (6, 8), with scattered CAR bacilli in the lower respiratory tract. No lesions were found in the upper or lower respiratory tract of guinea pigs experimentally inoculated with CAR bacillus from an infected rat (14, 20).

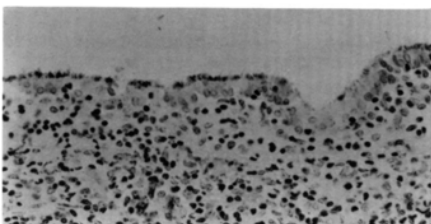


Figure 2. This bronchial section has been stained with the Warthin-Starry silver method. Numerous dark bacteria among the normally pale-staining cilia cause the entire layer to stain darkly.

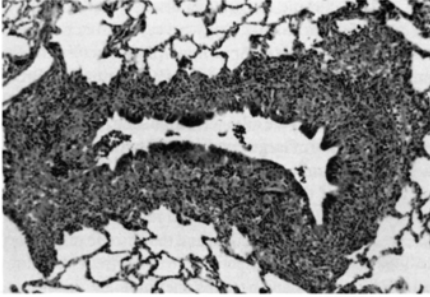


Figure 3. Low-magnification photomicrograph of lung from a rat infected with CAR bacillus. A dense infiltration of lymphocytes completely surrounds the bronchus in the center of the section.

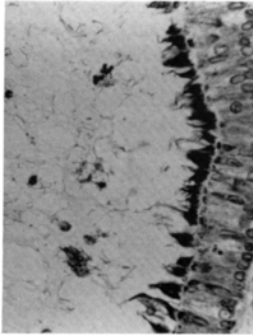


Figure 4. Photomicrograph of a bronchial section from a rat naturally infected with CAR bacillus. Numerous bacilli are crowded among the cilia at the edge of the section. The mucus-filled lumen also contains a few leukocytes.

Diagnosis

Diagnosis of CAR bacillus infection is based on identification of the filamentous organism among the cilia of the respiratory tract, using stains such as Warthin-Starry, Grocott methenamine silver, or immunoperoxidase (2, 5, 9). If infection is suspected, histologic examination of the trachea is necessary as the organism has been seen here before its colonization of bronchi and bronchioles (5). Serologic tests may offer the most efficient method of initial screening for CAR bacillus-infected colonies. The specificity of CAR bacillus serologic tests has not been directly addressed, but their use in experimental infectivity studies has not resulted in false positive values from CAR bacillus-free animals. Serologic testing combined with histologic examination of the respiratory tract may provide the most complete approach to definitive diagnosis at this time. Serologic tests available at commercial animal diagnostic laboratories include the ELISA (3, 7, 11) and IFA test (15).

Recommendations for serologic surveillance depend on the animal species, husbandry practices and population turnover. The optimum age of rodents for serologic screening of colonies is 10-14 weeks (3, 15, 16). From diagnostic and experimental data, pups of infected dams will have high antibody titers at three weeks (from passive antibody transfer), low to no titers at six to eight weeks as maternal antibody begins to wane, and then peaking titers at twelve weeks as active infection begins (3). If an epizootic is suspected,

rodents can be surveyed by three to four weeks after the onset of clinical disease, since seroconversion can be detected by two weeks post inoculation in rat and mouse infectivity studies (3, 14-16).

No information has been published on the ability of sentinel rodents to contract CAR bacillus through contact with soiled bedding from cages containing infected animals. Natural transmission appears to be mainly via direct contact with an infected animal or contaminated fomites; airborne infection is not considered an important mode of transmission (14). Infection of young sentinels may be enhanced by housing them with animals suspected of being infected.

Control

Caesarian derivation may be effective in eliminating CAR bacillus, since it has not been found in the reproductive tract, including the ciliated portions (2). Rodents can be maintained free of the organism by housing them in filter-topped cages, barrier rooms, or conventional rooms in facilities where the organism is not present. As cage-to-cage transmission has been documented (5), equipment or animal care staff should not be exposed to contaminated bedding, feed or caging. The high prevalence of CAR bacillus infection in the rabbit suggests this animal may serve as a reservoir. *In vitro*, the CAR bacillus is susceptible to antibiotics such as sulfonamides (3), penicillin G, gentamycin, tylosin, chloramphenicol, neomycin and streptomycin (17). *In vivo*, antibiotic therapy is not known to be successful or recommended as a method of control.

Effects on Research

Effects of CAR bacillus on research are undocumented and under study. Infected rodents have abnormal tracheobronchial cellular morphology and an increased lung lymphocyte population, raising concerns about their suitability in respiratory, immunology, carcinogenicity and physiology studies. The presence of the organism among the cilia on airway epithelium has been speculated to restrict normal ciliary movement (4). Electron microscopy shows loss of cilia in areas of dense colonization (18). If ciliary function is altered through ciliastasis or loss of cilia, host respiratory response to pharmacologic or infectious agents might be impaired.

Summary

The cilia-associated respiratory (CAR) bacillus is a novel bacterium morphologically similar to "gliding bacteria" that can only be cultivated *in vitro* in mammalian cell systems. It colonizes the airways of rodents and rabbits by nestling among the cilia of the respiratory epithelium. It is generally found in animals infected with respiratory viruses and/or *Mycoplasma*, but its ability to contribute to disease severity is still unclear. Although natural infection is usually subclinical, CAR bacillus has been reported to cause respiratory lesions in rodents free of common respiratory pathogens. No clinical or definitive morphologic signs of disease have been observed in infected rabbits. Although the bacillus is not convincingly established as a pathogen, its ability to colonize the entire respiratory tract raises concerns about its presence in research animals.

The epizootiology of the CAR bacillus is largely unknown. The rate of natural infection in rabbits appears to be high, suggesting that these animals may serve as a reservoir. Infection in conventional rodent colonies appears to be correlated with viral and mycoplasmal infections, but is not necessarily dependent on coinfection with other microbes. Serologic assays are probably the most efficient method of initial screening. Definitive diagnosis must be based on identification of argyrophilic filamentous bacteria in the cilia of airway epithelium, with or without the presence of respiratory lesions. Serologic screening followed by histologic examination is currently the most complete approach to identifying infected colonies.

Control measures are not well-defined. Screening animals for CAR bacillus infection before or during quarantine is necessary to prevent introduction of the organism into animal facilities and rooms. Since transmission is by direct contact with infected animals and possibly fomites, steps must be taken to prevent exposure of animals, equipment and animal care staff to contaminated bedding, feed or caging. Complete isolation of infected animals and caesarian rederivation may be applicable when animal replacement is not feasible.

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