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Monitoring of Rat Colonies for Antibodies to CAR Bacillus

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Introduction

The cilia associated respiratory (CAR) bacillus is a respiratory pathogenic bacterium of various animal species including mice (*Griffith et al.*,1988), rats (*Van Zwieten* 1980) and rabbits (*Waggie et al.*,1987). Guineapigs and hamsters (*Matsushita et al.* 1989; *Shoji-Darkey et al.*, 1991) are susceptible to experimental infection.

CAR bacillus infection can be diagnosed by histology (*Ganaway et al.*, 1985), culture (*Schoeb et al.*, 1993), PCR (*Cundiff et al.*,1994) and by various serological assays such as the indirect immunofluorescent antibody assay (IFA) (*Matsushita et al.*, 1987) and the enzyme-linked immunosorbent assay (ELISA) (*Ganaway et al.*, 1985). We investigated the occurrence of the bacterium by measuring antibodies to CAR bacillus antigen in sera from 10 *M.pulmonis* infected experimental and 10 *M. pulmonis* - free breeding colonies of rats.

Materials and Methods

Sera

Sera were obtained from 20 rat colonies. 73 Sera were from 10 *Mycoplasma pulmonis* ELISA positive colonies (all NL), which were also infected by one or more (non)respiratory viruses (data provided by Dr. J.T.M. van der Logt, ICLAS reference centre for rodent viruses, Nijmegen, the Netherlands). As none of these colonies were strictly barrier-maintained these were considered conventional. 183 Sera were obtained from 10 commercial and institutional, hysterectomy-derived barrier-maintained (SPF) breeding colonies (F, FRG, GB, NL) that were reported to be free from infection by *M.pulmonis* and other pathogenic microorganisms. (Histo)pathological examinations for CAR bacillus infection were not included in this study.

ELISA

Antibodies to *M.pulmonis* and CAR bacillus were determined by ELISA as described for *Streptobacillus moniliformis* (*Boot et al.*, 1993) using antigens of *M.pulmonis* (ICLAS reference centre for rodent viruses, Nijmegen, the Netherlands) and CAR bacillus (Harlan Olac Ltd., Blackthorn, Bicester, England). The ELISA was considered positive if the optical density (OD) value (extinction) of the 1 : 50 serum dilution exceeded the mean + 3 SD of the mean of the ODs in the sera of rats from *M.pulmonis* and CAR bacillus free SPF colonies. Positive and negative control sera were run in each test. The CAR bacillus ELISA was previously evaluated by comparing ELISA with histopathology in experimental infections using CAR bacillus infected tracheal scrapings from wild *R.norvegicus* and in transmission studies mimicking natural infection in WU rats (*Thuis et al.*, 1998).

Statistical analysis

Differences between groups were evaluated by Fischer's exact test using the Epistat statistical program run on a personal computer.

Results

All conventional rat sera were (again) positive by the *M.pulmonis* ELISA (Table 1). Six of the 10 conventional experimental colonies contained rats showing antibodies to the CAR bacillus antigen; the 26 CAR bacillus positive samples comprised 58 % of the samples from these 6 colonies and 36 % of the Mp+ samples. Antibodies to CAR bacillus and *M.pulmonis* were not detected in any

Table 1. ELISA antibodies to CAR bacillus in conventional *M. pulmonis* infected and in SPF colonies of rats

		Conventional colonies		SPF colonies	
		antibody ELISA		antibody ELISA	
colony	strain	*M. pulmonis*	CAR bacillus	colony	*M. pulmonis* & CAR bacillus
1	Wistar	5/5*	1/5	11	0/20
2	LEW/PVG	10/10	8/10	12	0/09
3	?	10/10	0/10	13	0/10
4	Wistar	7/7	3/7	14	0/32
5	Wistar	4/4	0/4	15	0/40
6	BN	6/6	1/6	16	0/28
7	Wistar	4/4	0/4	17	0/16
8	*R.norvegicus*	7/7	6/7	18	0/12
9	?	10/10	7/10	19	0/08
10	Wistar	10/10	0/10	20	0/08
		73/73	26/73		0/183

* positive/examined

of the 183 samples from the 10 SPF rat colonies. The percentage of CAR bacillus seropositive conventional rats was significantly higher than that of SPF rats (Fischer's exact test, $p < 0.001$).

Discussion

The increased use of SPF animals has decreased the occurrence of *chronic respiratory disease (CRD)* in rats. CRD has been frequently associated with *Mycoplasma pulmonis* infection *(Schoeb et al.*, 1996) and rather recently also CAR bacillus infection has been implicated in CRD (*Ganaway et al.*, 1985).

Natural CAR bacillus infection without *M.pulmonis* infection may be rare as Schoeb et al. (1997) found 15 of 16 primary rat CAR bacillus isolates only to be free from *M.pulmonis* infection. This finding is relevant to the interpretation of studies on the pathogenesis of CRD in rats, as lung lesions observed in experimental infections may have been the result of dual infection. Schoeb's findings also suggest that *M.pulmonis* might be present as a contaminant in the CAR bacillus antigen used in serological studies. Our study was not troubled by such a contamination as *M.pulmonis* positive sera were not invariably CAR bacillus positive (Table 1).

FELASA recommends to monitor colonies for CAR bacillus infection only when clinical signs or pathological observations suggest infection by the bacterium (*Kraft et al.*, 1994. *Rehbinder et al.*, 1996). As no antibodies to CAR bacillus antigen (indicating infection) were detected in any of the samples from the 10 barrier-maintained breeding colonies, the recommendations seem reasonable for well managed SPF colonies of rats. CAR bacillus infection (as suggested by seropositive animals) may however be present in colonies that should be considered as conventional as no specific preventive measures were in operation and infections by viruses (data not shown) and *M.pulmonis* were present. It might be argued that our results are biased by using *M.pulmonis* infection as a criterion for selecting conventional colonies but sera from conventional *M.pulmonis* uninfected rat colonies were not available. *M.pulmonis* infection is not invariably accompanied by CAR bacillus infection: the absence of CAR bacillus infection in 4 *M.pulmonis* positive colonies might partly be due to differences in the ease of transmission of both microorganisms. Vertical transmission has not been documented for CAR bacillus. Vertical transmission has however been found for *M.pulmonis* (*Ganaway et al.*, 1973) but (as expected) we did not detect the infection in SPF colonies. Horizontal transmission of CAR bacillus seems to be difficult, but no studies have been found comparing the ease of horizontal transmission of CAR bacillus and *M.pulmonis* directly.

In conclusion: our data suggest that CAR bacillus infection will only rarely be found in hysterectomy-derived, barrier-maintained (SPF) colonies of rats but may be encountered in less well protected (conventional) colonies. The postmortem examination of rats showing signs of CRD should include a search for CAR bacillus infection.

Summary

The cilia associated respiratory (CAR) bacillus is a respiratory pathogenic bacterium of rats and other species of animal. We determined, by enzyme-linked immunosorbent assay (ELISA) antibodies to CAR bacillus antigen in sera from 20 colonies of rats. Six out of 10 *Mycoplasma pulmonis* ELISA positive experimental colonies contained CAR bacillus seropositive rats, comprising 26 out of 45 (58 %) samples. CAR bacillus infection was not diagnosed by ELISA in 183 samples from 10 *M.pulmonis* free SPF-rat breeding colonies.

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