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Short Communication

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Natural Cilia-Associated Respiratory Bacillus Infection in Rabbits Used for Elaboration of Hyperimmune Serum Against *Mycoplasma* sp.

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With 3 figures

Summary

Cilia-associated respiratory (CAR) bacillus was identified in lung lesions of rabbits used for elaboration of hyperimmune serum against *Mycoplasma mycoides* ssp. *capri* (Mmc). Numerous Warthin Starry (WS) positive filamentous bacteria aligned perpendicularly to the surface of bronchial epithelial lining were observed. Immunoperoxidase staining of these bacteria was detected using a serum anti-rabbit CAR bacillus. Ultrastructural morphology corresponds to that of CAR bacilli previously reported in rabbits. The desirability of monitoring laboratory rabbits for CAR bacillus infection as part of the health programme is reinforced, especially in rabbits used for raising sera against respiratory pathogens of animal species in which CAR bacillus infection has been described. This is the first report of natural CAR bacillus infection in rabbits in Europe.

Introduction

Cilia-associated respiratory (CAR) bacillus is a descriptive term for unclassified, gram-negative, filamentous bacteria that colonize the respiratory epithelium of laboratory and wild rats (VAN ZWIETEN et al., 1980; MACKENZIE et al., 1981; GANAWAY et al., 1985; ITOH et al., 1987; BROGDEN et al., 1993), mice (MATSUSHITA et al., 1989), rabbits (WAGGIE et al., 1987; KURISU et al., 1990; CUNDIFF et al., 1995), cattle (HASTIE et al., 1993), pigs (NIETFELD et al., 1995), goats and lambs (FERNÁNDEZ et al., 1995, 1996). These bacteria are approximately the same length and diameter as cilia and lie parallel to cilia, being histologically detected with silver staining techniques such as the Warthin Starry (WS) method (VAN ZWIETEN et al., 1980; GANAWAY et al., 1985; KURISU et al., 1990; SHOJI-DARKYE et al., 1991; FERNÁNDEZ et al., 1996).

Natural infections in rabbits have been associated with hyperplasia of the laryngeal, tracheal and bronchial epithelia, loss of cilia and infiltration of heterophils, lymphocytes and plasma cells in the lamina propria, with much less severe lesions than those reported in rats (WAGGIE et al., 1987; KURISU et al., 1990). Rabbits intranasally inoculated with the SMR strain of rat CAR bacillus did not develop inflammatory lesions, but did seroconvert (MATSUSHITA et al.,

1989; SHOJI-DARKYE et al., 1991). On the other hand, isolates of CAR bacillus from rabbits appeared to be less virulent in inoculated mice than rat CAR bacillus, resulting in limited colonization, undetectable humoral immune response, and no demonstrable lesions (CUNDIFF et al., 1994).

In the course of the investigations in which CAR bacillus was described in goats for the first time (FERNÁNDEZ et al., 1995, 1996), the immunohistological study using a specific hyperimmune serum raised in rabbits against the reference strain of *Mycoplasma mycoides* ssp. *capri* (Mmc) (PG3, supplied by J. B. POVEDA) revealed a positive filamentous immunoreaction in the ciliated respiratory epithelium of WS (+) goats. The negative immunostaining at the same localizations using other anti-Mmc sera, the ultrastructural demonstration of CAR bacillus in these goats and the absence of mycoplasmas in these areas, allowed the presence of anti-CAR bacillus antibodies to be suspected in this rabbit serum anti-Mmc.

A retrospective histological, histochemical, immunohistological and ultrastructural study of the lungs of the rabbits employed in the anti-Mmc serum preparation was made to demonstrate the presence of CAR bacillus in these rabbits.

Materials and Methods

The serum anti-Mmc had been obtained following the procedures described by SENTERFIT (1983). Seven 6-week old female New Zealand White rabbits weighing 1.30–1.50 kg had been inoculated subcutaneously with a suspension of the Mmc isolate in phosphate buffered saline (PBS) and incomplete Freund's adjuvant. Serological and/or bacteriological studies on these rabbits had been performed for respiratory pathogens and they were free of *Pasteurella multocida*, *Bordetella bronchiseptica*, *Streptococcus zooepidemicus*, and *Mycoplasma pulmonis*. The rabbits had been housed in stainless steel cages with wire mesh bottoms (two rabbits/cage), fed commercial pellets and allowed free access to tap water. The animal facility had been maintained at 23–25°C.

Retrospectively, lung sections of paraffin-embedded material from these rabbits were studied with the haematoxyline–eosine (H&E), WS, Gram and periodic acid-Schiff (PAS) methods.

For the immunohistological study a serum anti-rabbit CAR bacillus raised in guinea pigs determined to be free of antibody to CAR bacillus by use of the enzyme-linked immunosorbent assay (ELISA) (CUNDIFF et al., 1994) was used. An immunoperoxidase technique based on the labelled streptavidin biotin (LSAB) method and 3-amino-9-ethylcarbazole (AEC) as substrate was carried out. The primary antibody was applied at dilutions ranging from 1:50 to 1:1000. A serum anti-rat CAR bacillus raised in mice (kindly supplied by S. MATSUSHITA) was also used at the same dilutions. Identical slides were processed simultaneously using negative control serum.

The ultrastructural study was carried out from the paraffin-embedded tissues following procedures previously described (WANSON and DROCHMAN, 1968). Selected 90 nm sections were stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (TEM).

Results

No clinical signs such as coughing, sneezing or rale had been noted in any of the rabbits. None of the animals had exhibited gross lesions after routine macroscopic examination. Histologically CAR bacilli were demonstrated in three of seven rabbits. The ciliated borders of epithelial cells on a few bronchi in these animals were more basophilic than normal, and stained strongly with PAS. In lung sections stained with the WS method, there were numerous argentophilic filamentous bacteria aligned perpendicularly to the surface of the bronchial epithelial lining, situated among the cilia. Lung lesions ranged from a mild to severe peribronchial lymphoplasmocytic hyperplasia (Fig. 1). The bronchial epithelium was normal to slightly hyperplastic, with a few areas of loss of cilia associated with slight lymphoplasmocytic infiltration in the lamina propria. No histological lesions were detected in the remaining rabbits.

Immunoperoxidase staining of bacteria lining the ciliated bronchial epithelium from the three rabbits found by WS staining to contain CAR bacillus-like bacteria was observed using the anti-rabbit CAR bacillus guinea pig serum at a dilution of 1:500 (Fig. 2). A very weak immunopositive reaction was observed in these localizations using the serum anti-rat CAR bacillus at dilutions ranging from 1:50 to 1:500.

By TEM, long thin bacteria, similar in size and shape to the cilia, were found on the

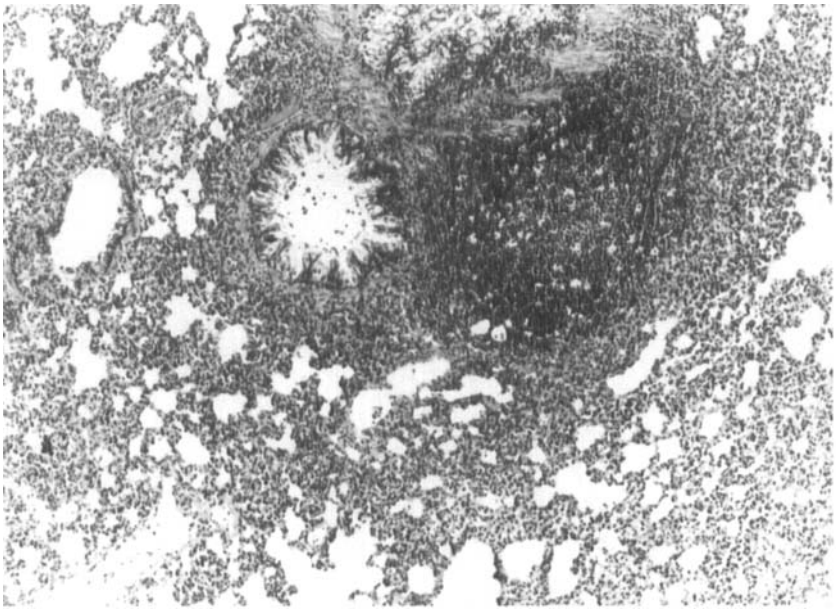

Fig. 1. Marked peribronchial lymphoplasmocytic hyperplasia (H&E, 10 ×)

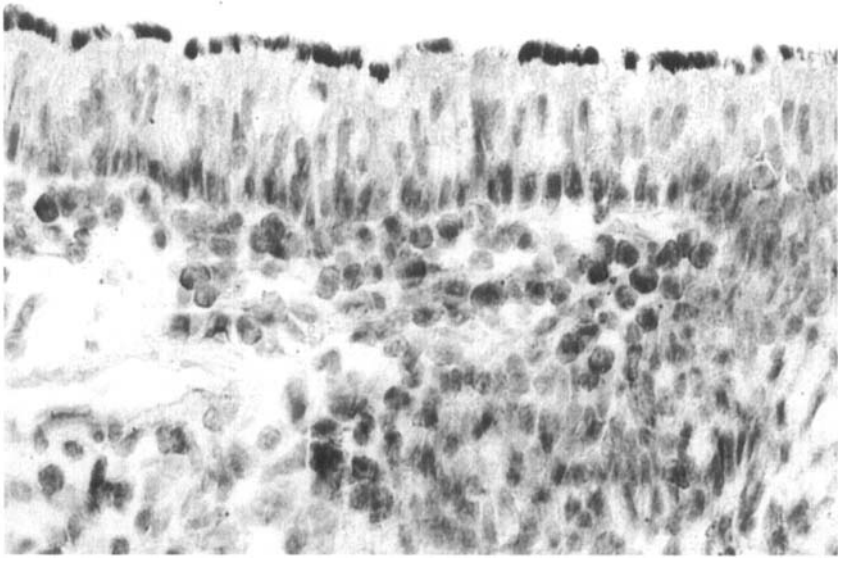

Fig. 2. Immunoperoxidase labelling (LSAB) of rabbit CAR bacilli lining the ciliated bronchial epithelium (Mayer's haematoxyline counterstain, 40 ×)

surface of ciliated epithelial cells. The ends of the CAR bacilli attached to the host cells, sometimes of bulbous form, were 0.15–0.19 μm in width, being thinner than the rest of the bacterial body (0.20–0.25 μm in width). The length of bacilli varied from 3 to 5 μm (Fig. 3).

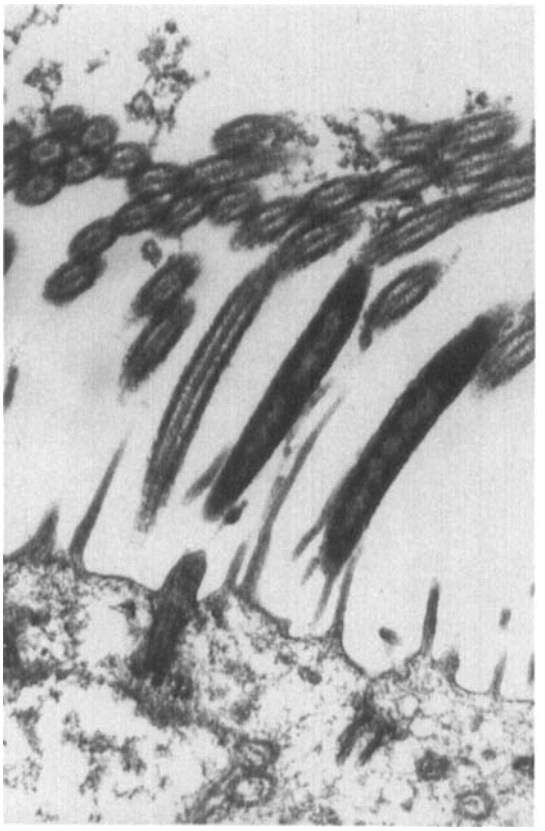

Fig. 3. Filamentous bacilli are closely applied to the cilia and to the epithelial cell membrane (uranyl acetate counterstain, 17 000 ×)

Discussion

Histological lung lesions in these WS (+) rabbits were less severe than those reported in naturally infected and experimentally inoculated rabbits with CAR bacillus by WAGGIE et al. (1987), but were similar to those described by KURISU et al. (1990). Different pathogenicity of rabbit CAR bacillus strains have been suggested previously (KURISU et al., 1990). Ultrastructural morphology of these bacteria was very similar to that of CAR bacilli previously reported in rabbits (WAGGIE et al., 1987; KURISU et al., 1990).

The strong immunoperoxidase labelling of CAR bacilli using the anti-rabbit CAR bacillus guinea pig serum and the very weak immunoreaction with the serum anti-rat CAR bacillus confirms that both bacteria are antigenically different. Recently, studies of characterization of CAR bacillus in rabbits and rats and analysis of the 16S RNA gene sequence have indicated that these two microorganisms are distinctly different, with only 48.8% sequence homology (CUNDIFF et al., 1995): rabbit CAR bacillus is most closely related to the genus *Helicobacter* (CUNDIFF et al., 1995), whereas rat CAR bacillus is more closely related to organisms within the genus *Flavobacterium* (SCHOEB et al., 1993).

To the authors' knowledge, natural CAR bacillus infections in rabbits have only been described in the US (WAGGIE et al., 1987; CUNDIFF et al., 1995) and Japan (KURISU et al., 1990). This study is therefore the first report of natural CAR bacillus infection in rabbits in Europe.

On the other hand, these results reinforce the desirability of monitoring laboratory rabbits for CAR bacillus infection as part of the health assessment programme, especially in rabbits

used for raising sera against respiratory pathogens of animal species in which CAR bacillus infections have been described. Serological tests for immunoglobulin (Ig) G antibody to rabbit CAR bacillus antigens by ELISA could be successfully used for diagnosis (CUNDIFF et al., 1995), although further studies are needed to establish the specificity and sensitivity of these tests for detection of the infection in laboratory rabbits.

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