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Chronic inflammatory lung lesions in rabbits free of known respiratory pathogens

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INTRODUCTION

Chronic inflammatory lung lesions were frequently observed during a health monitoring study of purpose bred laboratory rabbits (*Feinstein & Rehbinder,* 1988). In that investigation many of the rabbits were infected with B.bronchiseptica (*Uzal et al,* 1989), but pulmonary changes were also noticed in numerous rabbits that were free of known respiratory pathogens. The aim of the present study was to provide a description of lung lesions found in 58 rabbits that were free of agents commonly associated to respiratory disease, such as *Berdetella bronchiseptica, Pasteurella spp., Klebsiella spp., Toxoplasma gondii,* and lung worms. The etiology of the lung lesions here described remains unknown.

MATERIAL AND METHODS

Animals:

The material examined was obtained in a previous investigation (*Feinstein & Rehbinder,* 1988). Lung tissues were collected from 58 purpose bred, laboratory rabbits, of both sexes, of varying age (young and adult), and with body weights ranging from 0.7 till 3.0 kg. Most of the rabbits were of the New Zealand white breed, born and maintained in 8 colonies under various conventional conditions, but 9 of the rabbits were of the NZW/Swiss Hare breed and originated from a colony provided with strict barriers. Information concerning stables and sanitary conditions at the rabbitries is presented elsewhere (*Feinstein & Rehbinder,* 1988). The rabbits were clinically healthy and they were shown to be free from *Bordetella bronchiseptica, Pasteurella spp., Klebsiella spp.,* and from other bacterial pathogens. The rabbits were also found negative for *Toxoplasma gondii, Encephalitozoon cuniculi* and lung worms. The bacteriological, parasitological, and virological procedures were conducted following the STANDARD OPERATIONAL PROCEDURES of The National Veterinary Institute, Uppsala, where diseases of laboratory animals are regularly diagnosed as a part of health monitoring and diagnostic activities.

Procedures:

The rabbits were sacrificed with 0.5 ml/kg Fluanison – Fentanil (Hypnorm, Jansen Pharmaceutica), i.m., followed by 60 mg/kg pentobarbitone (Mebumal, ACO Läkemedel), intracardially. A complete necropsy was immediately performed and samples were obtained for diverse studies, such as bacteriology, parasitology, virology and histology, as described elsewhere (*Feinstein & Rehbinder,* 1988). In order to minimize the occurrence of autolytic changes the samples for histology were collected and fixed as rapidly as possible. Fragments of lung tissues, measuring approximately $1 \times 1{,}5 \times 0{,}5$ cm, were randomly obtained, as macroscopical changes were not noticed, and fixed by immersion in 10% buffered neutral formalin for 24 hs. Following routine processing and embedding in paraffin wax, 4 µm thick sections were cut and stained with hematoxylin & eosin. Selected sections were also stained with Giemsa, Gram, PAS and van Gieson.

RESULTS

The fixation procedures used did not lead to artifacts, such as lung collapse. Each and every one of the rabbits examined exhibited chronic inflammatory lung changes. Fiftyseven rabbits (98.2%) displayed lesions diagnosed as focal chronic interstitial pneumonia (FCIP). Usually, FCIP appeared as a variable number of dis-

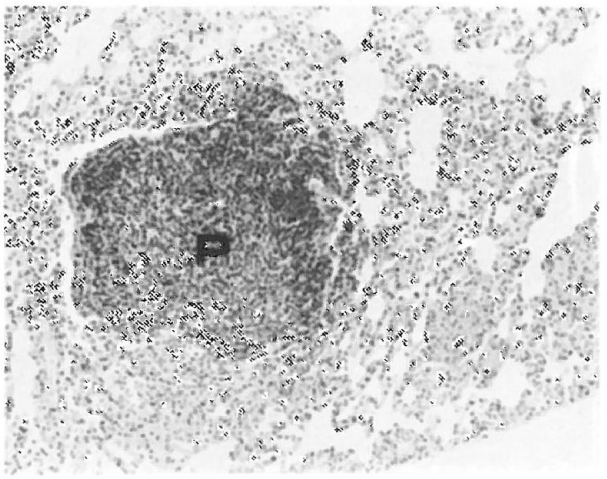

Fig. 1: Focal chronic interstitial pneumonia. A zone (P) displaying mixed leukocytic infiltration with predominance of lymphoid cells. Giemsa, ×40.

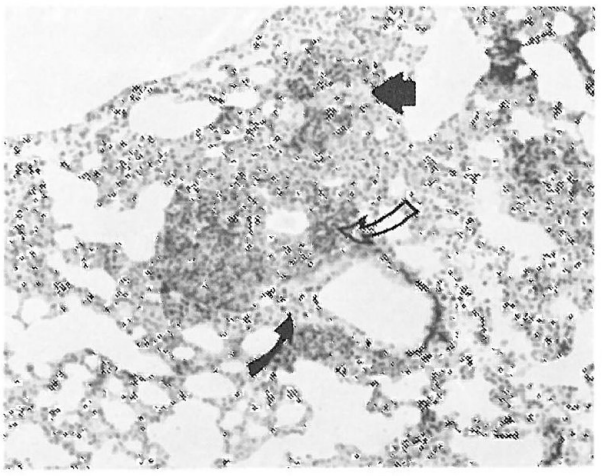

Fig. 3: Chronic bronchiolitis. A zone exhibiting marked leukocytic infiltration at the level of a bronchioloalveolar junction (curved dark arrow). Notice severe perivascular cuffing (curved hollow arrow) and consolidation (short arrow). Giemsa, ×40.

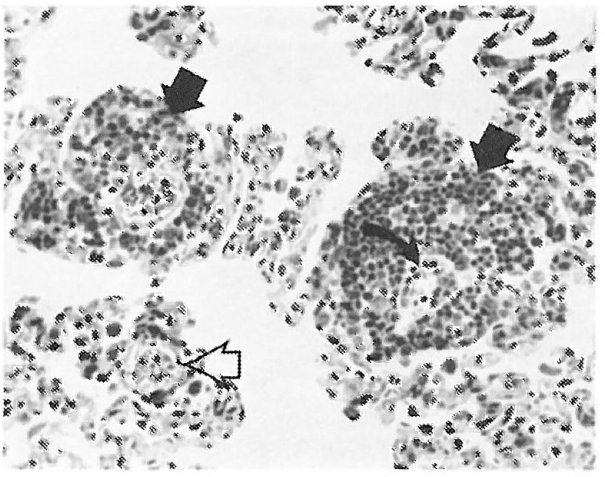

Fig. 2: Vascular changes. Marked perivascular infiltration of, mainly, lymphoid cells (thick arrows). Notice thickened vascular wall due to vasculitis in one of the vessels (curved arrow). A hollow arrow points at a normal vessel. Giemsa, ×100.

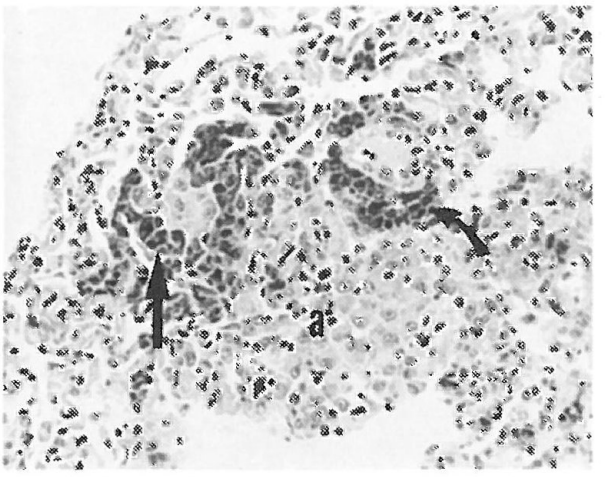

Fig. 4: Focal chronic interstitial pneumonia displaying a thickened alveolar septum due to, mainly, infiltration of lymphoid cells (straight arrow), alveoli filled with large macrophages (a), and perivascular cuffing arround an arteriole (curved arrow). Giemsa, ×100.

crete, irregularly shaped areas that varied markedly in size, reaching a maximal diameter of approximately 500 µm (Fig 1). FCIP exhibited a patchy distribution, but in some few rabbits the lesions were so extensive that most of the lung tissue examined was involved. The changes ranged from thickened alveolar septae to consolidation, which was frequently associated with perivascular infiltrations and also with vasculitis (Fig 2).

Occasionally, perivascular infiltration was so severe that adjacent tissues were markedly compressed. Perivascular infiltrates consisted mainly of lymphocytes and a low number of plasma cells (Fig 2). In pneumonic areas the composition of the exudate varied between different zones of one and the same section. Certain areas displayed a mixed leukocytic infiltration, with macrophages, monocytes, and mononuclear cells (Fig 4), whereas other zones exhibited a more homogeneous infiltrate in which either macrophages and monocytes, or mono-

nuclears, were the dominant feature (Fig 1). The number of granulocytes was low. Alveolar macrophages were conspicuous and not uncommonly they fused and formed large syncytial multinucleated cells. However, foreign bodies, such as plant tissues, or features of an organized granuloma were not observed. Numerous rabbits exhibited hyperplasia of cells resembling type II pneumocytes (epithelialization). Severe epithelialization produced occlussion of alveoli. In the lesions mitotic figures constituted a usual finding. Alveolar septae frequently displayed a moderate proliferation of fibrous tissues and deposition of a fibrilar substance that was stained metachromatically with Giemsa. Numerous rabbits also exhibited a mild chronic inflammation of the airways, most commonly confined at the level of the bronchiolo-alveolar junction (Fig 3). These changes were characterized by infiltration of macrophages and mononuclears, which were located both around and in the lumen of the airways. Sections stained with Giemsa, Gram and PAS did not reveal micro-organisms.

DISCUSSION

Pulmonary lesions constitue a most frequent problem in rabbits (*Feinstein & Rehbinder,* 1988). We have observed chronic inflammatory lung changes in clinically healthy rabbits that were free of known respiratory pathogens. Strawbridge (1960), in a study of spontaneous pulmonary emphysema of rabbits observed subacute or chronic interstitial pneumonia in 84 per cent of cases. In the study of *Strawbridge* (1960) *Gram* or *Giemsa* stained sections did not reveal microorganisms and bacteriological studies were not carried out. In rabbits, pulmonary disease is usually associated with *Pasteurella multocida, Bordetella bronchiseptica,* and less frequently with other agents, such as *Klebsiellae, Staphylococci, E. coli, Salmonella,* and *Listeria* (*Oldenburg et al,* 1972, *Flatt,* 1974, *Bemis,* 1986, *Lebas et al,* 1986, *Uzal et al,* 1989). None of these organisms was isolated from the lungs included in the present study. Moreover, the lungs were negative for other bacteria, as well as for *mycoplasma spp.* Nevertheless, the possibility remains that if bacterial organisms were present, albeit in exeedingly low numbers, they could have escaped detection by the cultural procedures used. In addition, if bacteria were irregularly distributed in the lung tissues, it is also conceivable that some of the cultural samples obtained derived from areas that did not contain microorganisms. In order to examine for this possibility, further studies are in progress. The present rabbits were tested and found negative also for antibodies against the protozoa *Toxoplasma gondii* and *Encephalitozoon cuniculi,* and lesions attributable to these parasites were not noticed. Finally, parasitological and histopathological examinations did not reveal lung worms. *Ostler* (1961), considered that in enzootic pneumonia of rabbits bacterial isolations could represent a secondary infection following a primary virus or a protozoan disease, and that in certain adult rabbits, the lesions suggested a »proliferative virus-type pneumonia«. Viruses are frequently associated with respiratory disease. However, presently only one virus has been linked to the genesis of naturally occurring pneumonia in rabbits. Recently, a *picornavirus*-associated hemorragic pneumonia has been described (*Liu et al,* 1984) but the clinical signs and the pathological changes characteristic of that disease are quite different from our findings. In other species, *reoviruses* induce a variety of lesions in several organs, including lungs (*Scott et al,* 1972, *Tyler & Fields,* 1986). *Reovirus* antibodies have been found in rabbits (*Rosen,* 1962) but we are not aware of any report on changes attributable to *reoviruses* in this species. In young dogs *Thompson et al* (1970) with *reovirus* produced alterations such as the presence of intra-alveolar syncytial giant cells and macrophages with vacuolated cytoplasm, and perivascular infiltrations of lymphoid cells, which resembled some of our observations in rabbits. Rabbits experimentally inoculated with *reoviruses,* however, remained clinically healthy and did not display lung changes (*Lou et al,* 1963). In other species, *adenoviruses* are frequently associated with respiratory disturbances, but inclusion bodies, a distinctive feature of *adenovirus*-in-

duced lesions (*Kunstyr et al,* 1984, *Dungworth,* 1985), were not noticed in the present study. A persistent infection of rabbits with a human *adenovirus type 5* was produced experimentally (*Reddick & Lefkowitz,* 1969, *Maré,* 1974), but changes attributable to this infection were not reported. *Caliciviruses* have been involved in respiratory disease (*Dungworth,* 1985), but to our knowledge *Caliciviruses* have not been isolated from rabbits. *Sendai,* a parainfluenza 1 virus which is widespread among colonies of mice, rats, hamsters and guinea pigs has not been recognized in rabbits.

The complex lesions that we noticed exhibited certain similarities with changes observed in rabbits after experimental infection by a *chlamydia* organism (*Flatt & Dungworth,* 1971), but such agents were not noticed in our material.

Pulmonary lesions could be produced also by inhalation of airborne contaminants such as fungal spores, particles from bedding or fodder, dust mites and their metabolites, and noxious gases like ammonia (*Clarke,* 1987).

Generally, deposition of particles in lungs gives rise to granulomas and severe proliferation of fibrous tissue, which was not the case in the presently investigated rabbits. In some of the colonies, long term exposure to ammonia could have played a certain role, but the best rabbitries were characterized by high hygienic standards and good ventilation of the stables, which excluded ammonia. Farmer's lung and chronic interstitial pneumonia in cattle is originated by exposure to spores of thermophilic actinomycetes (*Micropolyspora faeni* and *Thermoactinomyces vulgaris*) and moulds (*Asperigillus spp.*), which elicits an allergic alveolitis and bronchiolitis. Briefly, in cattle the lesions consist of bronchial and bronchiolar infiltrations of lymphocytes and plasma cells, thickened alveolar septae due to inflammatory infiltration, presence of granulomas composed of epithelioid and giant cells, and severe interstitial fibrosis (*Hogg,* 1982, *Dungworth,* 1985). Although we noticed some of these changes in our material, bronchial lesions and interstitial fibrosis were mild and organized granulomas did not occurr. It cannot be excluded, however, that, if present, the histological features of an allergic alveolitis in rabbits may differ from other species.

Chronic inflammatory lung lesions do constitute a very important problem of laboratory rabbits, and diverse pathogens are frequently found in association with these changes. However, we have now described pulmonary alterations in rabbits free of known respiratory pathogens. The changes mentioned do not constitute a normal feature of rabbit lungs. It appears unlikely that they were a consequence of the continuous stimulation from ubiquitous agents. Rabbits maintained under conventional conditions that were submitted at The National Veterinary Institute for health monitoring and diagnosis, at other occasions, did not exhibit lung changes. The etiology of the lesions found remains unknown. The environmental and hygienic conditions at the rabbitries were very heterogeneous, ranging from very good to bad (*Feinstein & Rehbinder,* 1988), but the lung changes observed in rabbits from the different groups were of a similar nature. This could indicate that the same factor producing the lesions was present at the various colonies. It is also possible that in rabbits diverse injuries could elicit a similar morphological lung response. Further studies are required in order to characterize these changes and to understand their pathogenesis.

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Summary

Chronic inflammatory lung lesions were studied in 58 purpose bred, clinically healthy rabbits that were found negative for *Bordetella bronchiseptica, Pasteurella multocida, Toxoplasma gondii, Encephalitozoon cuniculi,* and lung worms. The changes found consisted of focal chronic interstitial pneumonia and chronic bronchitis and bronchiolitis, which were frequently associated with perivascular infiltrations of mononuclear leukocytes and vasculitis involving small lung vessels. The etiology of these alterations remains unknown.

Sammanfattning
Kroniska inflammatoriska lungförändringar har undersökts hos 58 destinationsuppfödda, kliniskt friska kaniner, fria från infektion med *Bordetella bronchiseptica, Pasteurella multocida, Toxoplasma gondii, Encephalitozoon cuninculi* och lungmask. De funna förändringarna bestod av fokal kronisk interstitiell pneumoni och kronisk bronkit och bronkiolit, ofta förbundna med perivaskulära infiltrat av mononukleära leukocyter och vaskulit hos små lungkärl. Etiologien till dessa förändringar har förblivit okänd.

Yhteenveto / K. Pelkonen
Tässä työssä oli tutkittu 58:sta tavoitekasvatetusta kliinisesti terveestä kanista kroonisia tulehdusvaurioita keuhkoista. Yhdessäkään kanissa ei ollut osoitettavissa Bordetella bronchiseptica, Pasteurella multocida, Toxoplasma gondii, Encephalitozoon cuniculi tai keuhkomatoja. Havaittuja muutoksia olivat seuraavat: pesäkkeinen interstitiaalipneumonia sekä krooninen bronkiitti ja bronkioliitti, joihin usein liittyi mononukleaaristen leukosyyttien perivaskulaarinen infiltraatio ja pienten keuhkosuonten tulehdus. Näiden muutosten etiologia jäi epäselväksi.

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