Hypersensitivity Pneumonitis: Experimental Production in Calves with Antigens of Micropolyspora faeni

B. N. Wilkie*

ABSTRACT

Pneumonitis was induced in calves by exposure to aerosols of Micropolyspora faeni with or without prior sensitization of the animals by subcutaneous injection of antigen. The pneumonitis primarily involved centrolobular areas and was characterized by alveolar septal thickening and loss of air space by cellular infiltration. Vasculitis and focal haemorrhage occurred in certain individuals and haemoproteineaceous exudate appeared within septa and alveolar lumina. The pneumonitis was compared with human farmer’s lung, pneumonitis of housed cattle and other experimental hypersensitivity pneumonitides.

INTRODUCTION

Interstitial pneumonitis has been described in housed and pastured cattle in acute and chronic forms (2, 11). The disease of housed cattle has been compared with hypersensitivity pneumonitis of man (farmer’s lung) (14) and the immune response to the thermophilic actinomycete *Micropolyspora faeni* has been implicated in the pathogenesis (12, 20, 23). Experimental reproduction of allergic pneumonitis with antigens of *M. faeni* has been reported using guinea pigs and rabbits (10, 22).

The pathological changes in the lungs of calves exposed by aerosol to antigens of *M. faeni* are described here. Detailed description of clinical and immunological response is reported elsewhere (21) and shall be treated only briefly in the present communication.

MATERIALS AND METHODS

Experiments utilized healthy calves of several breeds ranging in age between four and ten weeks at the beginning of the study. Four calves were sensitized by a single subcutaneous injection of 30 mg soluble *M. faeni* antigen in Freund’s complete adjuvant (FCA) plus 10 mg in saline given intravenously. These and six additional normal calves were exposed for fifteen minutes at six weekly intervals to an aerosol generated from a 1% w/v aqueous suspension of washed, lyophilized ultrasonically disrupted insoluble *M. faeni* antigen (22). Two additional nonsensitized calves were each exposed on the same schedule to aerosols of distilled water.

Lung was obtained for biopsy before the

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*Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

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first and twenty-four hours after each subsequent aerosol exposure from all sensitized and from two nonsensitized calves. Respiratory response to aerosol exposure was observed and respiratory rate determined before and at intervals after exposure. Parameters of *M. faeni* — specific humoral and cell-mediated immune response were sequentially evaluated in all calves.

**Lung Biopsy**

Lung was obtained for biopsy by means of a trephine and compressed nitrogen-driven turbine drill according to the method of Steel (18). Calves were sedated with xylazine and the trephine introduced through a 1.0 cm skin incision made in the space between ribs 4 and 5 or 5 and 6 in the upper half of the thorax. Sequential interventions were made on alternate sides. All animals were killed by intravenous barbiturates or by electrocution 24 hours after the sixth aerosol exposure and their lungs submitted for pathological examination.

**Pathology**

At the time of necropsy lungs were examined for macroscopic lesions and portions of each lobe fixed in buffered formalin-saline for micropathological examination following sectioning and staining with haematoxylin-eosin (H & E), phosphotungstic acid haematoxylin, periodic acid Schiff, Van Gieson's method and Carbol Chromotrope R for eosinophils.

**ImmunoFluorescence**

Fluorescein isothiocyanate labelled anti *M. faeni*, anti bovine globulin and anti bovine complement were prepared as previously described (20) and used to stain cryostat sections of lung previously fixed for ten minutes in 5% acetic acid-ethanol and washed in neutral phosphate buffered saline.

**RESULTS**

**Clinical-Immunological Response**

All calves sensitized by systemic injection of *M. faeni* antigen responded to first aerosol exposure to homologous antigen given four to five weeks later. Respiratory rate was significantly elevated with onset zero to three hours postexposure with tachypnoea generally persisting into the three to ten hour interval. A similar pattern occurred following each subsequent exposure with elevation in the ten to 48 hour period seen especially following third, fourth and fifth exposures. A typical series of responses is shown in Fig. 1. Nonpresensitized calves either failed to respond upon first exposure (3/6), responded with slight tachypnoea (1/6) or responded qualitatively and quantitatively as if presensitized (2/6). Response became positive and then of either increasing or decreasing severity following subsequent weekly exposures (Fig. 1). Calves exposed to aerosols of water did not react with tachypnoea or dyspnoea or in any other observable manner.

Calves with tachypnoea were frequently dyspnoeic especially in the three to ten hour interval and they often had a dry cough and were reluctant to move or to eat. Dyspnoea was accompanied by harsh, loud lung sounds.

**Immune Response**

Exposure to *M. faeni* antigens was associated with rising serum and nasal antibody titres as determined by passive and indirect haemagglutination and with transient presence of peripheral lymphocytes capable of producing macrophage migration inhibition factor when cultured with *M. faeni* antigens (21). The macrophage migration inhibition test values were beneath mean normal by one week following either sensitization or first aerosol exposure in nonsensitized calves but had returned to preexposure values in both groups by the time of fifth aerosol exposure.

**Biopsy of Lung**

Lung tissue obtained for biopsy was found adequate for histological examin-
The trephine apparently entered the dorsal area of the cardiac lobe and the most frequent complication was penetration of the great vessels or heart which was usually uneventful. Severe haemorrhage into the airway was experienced on one occasion and although the calf survived it was excluded from the study.

**MACROSCOPIC PATHOLOGY**

Lesions were most apparent in the lungs of presensitized calves, three-quarters of which had firm dark red anteroventral lobes with individual dark apparently atelectatic lobules adjacent to normally aerated lobules in remaining lobes. Lungs of calves not previously sensitized were near normal in appearance, there being only minor foci of firm dark discoloured tissue especially in the roots of lobes. Both groups of aerosol exposed calves had 1-2 mm pale foci visible beneath the visceral pleura on the surface of all lobes. In all calves subjected to trephine lung biopsy small connective tissue tags occurred on the visceral

**Fig. 1.** Respiratory response of sensitized calf (above) and nonsensitized (below) to weekly aerosol (arrows) of *M. faeni* antigen. Divisions on the horizontal axis indicate two day intervals. Respiratory rate observations were made at irregular intervals to include the periods 0-3, 3-10, and 10-48 hours postexposure.
pleural surface and occasionally on aortic serosa.

**Microscopic Pathology**

Lung obtained for biopsy prior to exposure to aerosols was in every case considered free of remarkable microscopic lesions (Fig. 2) as were sections of lung obtained from calves repeatedly exposed to aerosols of water without prior sensitization with *M. faeni* antigen. Peribronchiolar and perivascular lymphoid nodules were present in lung of all calves regardless of treatment and were taken as a normal histological feature of lung in this group of calves.

Earliest histological changes occurred in pulmonary tissue obtained after the third aerosol exposure of presensitized calves. In these, minor alveolar septal thickening adjacent to terminal bronchioles and alveolar ducts was accompanied by exfoliation of macrophages into alveolar lumina with some intraseptal and intra-alveolar accumulation of lymphocytes, polymorphonuclear cells and proteinaceous exudate (Fig. 3). Similar lesions were recognized with frequency and severity in-

Fig. 2. Bovine lung obtained for biopsy prior to sensitization and aerosol exposure to antigen of *M. faeni*. H & E. X20.

Fig. 3. Bovine lung obtained for biopsy following sensitization and four exposures to aerosols of *M. faeni* antigens. Septal thickening with activation of alveolar epithelium. H & E. X20.

Fig. 4. Diaphragmatic lobe of calf exposed to six weekly aerosols of *M. faeni* antigen without prior sensitization. Marked septal thickening in alveolar duct region with loss of air space. H & E. X8.
increasing slightly until the last biopsy. Lung of calves not previously sensitized developed only mild lesions detected by biopsy and consisting mainly of slight septal thickening.

Lung obtained at necropsy generally confirmed impressions obtained by biopsy. However changes were more extensive and severe than had been anticipated from ante mortem samples. Sensitized calves were clearly more severely affected than were those exposed to antigen without prior sensitization.

The most frequently observed lesion in all animals was moderate to marked thickening of alveolar septa especially in the central lobular area with partial diminution of air space in some locations (Fig. 4). Thickened septa contained macrophages, lymphocytes, neutrophils and eosinophils (Fig. 5). Disruption of septa was frequently seen especially in peripheral areas of lobes. Proteinaceous exudate containing alveolar macrophages occasionally occupied alveolar lumina and erythrocytes were often free in air spaces or phagocytized by macrophages (Fig. 6). The most severe lesions were in anteroventral lobes which had appeared atelectatic. In these, the air space

Fig. 5. Diaphragmatic lobe of calf exposed to six weekly aerosols of M. faeni antigen without prior sensitization. Septal thickening including neutrophils, eosinophils, macrophages and lymphocytes with proliferation and exfoliation of alveolar epithelial cells. H & E. X50.

Fig. 6. Cardiac lobe of sensitized calf exposed to six weekly aerosols of M. faeni antigen. Proteinaceous and leucocytic exudate within alveolar space. H & E. X50.

Fig. 7. Apical lobe of calf exposed to six weekly aerosols of M. faeni antigen without previous sensitization. Terminal bronchiole containing alveolar macrophages, lymphocytes and neutrophils. H & E. X50.
was obliterated by alveolar macrophages, polymorphonuclear leukocytes and extravasated blood. Bronchioles and alveolar ducts were often occluded by purulent exudate or alveolar macrophages and the epithelium had undergone squamous metaplasia (Fig. 7). Eosinophils were found with varying frequency from area to area of a section but were not predominant in any location. Segmental hyaline change occurred in walls of small and medium blood vessels and platelet thrombi were occasionally seen in septal capillaries. Severe vasculitis involving medium sized vessels occurred occasionally and was especially common in two calves which had not been sensitized prior to exposure to aerosols. Focal haemorrhage occurred adjacent to injured vessels (Fig. 8) and neutrophils, eosinophils and lymphocytes were numerous within vessel lumina and walls as well as in perivascular areas. Proteinaceous exudate filled septae and alveoli adjacent to foci of vasculitis.

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Specific staining for M. faeni and bovine complement was not observed in lung sections. Specific anti bovine globulin staining was observed in the absence of M. faeni and complement-specific fluorescence and was therefore pathogenetically insignificant.

DISCUSSION

Hypersensitivity pneumonitis has been produced experimentally by sensitization with antigens of the thermophilic actinomycete Micropolyspora faeni either by subcutaneous and intravenous injection followed by aerosolized antigen or by repeated aerosol exposure alone (21). The microscopic lung lesions observed resemble those described by Dungworth (4) following sensitization of cattle with ovalbumin and are similar to lesions seen in laboratory animals sensitized with antigens of M. faeni (10, 22) or with other antigens (9, 5, 15, 16).

While the pneumonitis described here may bear some resemblance to the human condition farmer's lung (17) and to pneumonitis seen in housed cattle (11, 14), which are both associated with immunological reaction to M. faeni antigen, the lesions observed cannot be considered characteristic enough to be pathognomonic of hypersensitivity pneumonitis. Vasculitis and focal haemorrhage observed in certain individuals strongly suggest an Arthus reaction and are not commonly seen in bovine pneumonitis of different etiology. Proof of occurrence of an Arthus reaction is however absent and supported only indirectly in the present study by temporal aspects of the clinical response and by histological evidence of vascular damage.

Absence of granulomata and of fibrosis are at variance with findings in both human and bovine hypersensitivity pneumonitis occurring under field conditions although lesions do resemble those reported by Barrowcliff (1) in an acute fatal case of farmer's lung which occurred in a 17 year old boy. The brevity and infrequency of exposure and the low concentration of antigen used in the present experiments most likely account for the moderate pneumonitis obtained when compared with bovine or human field cases of long standing.

Hypersensitivity pneumonitis induced by a variety of antigens has frequently been considered to be due to immune com-
plex mediated injury (8, 13) or to complement related cytotoxicity (3, 19, 20). The additional role of delayed hypersensitivity reactions has recently become apparent (3) and involvement of immediate hypersensitivity has also been suggested (8). Immunological, clinical and pathological observations on the present series of calves suggest involvement of immediate and delayed hypersensitivity but with the greatest part of both clinical and histopathological signs likely attributable to humoral antibody-mediated reactions (21).

Histological evidence exists for vasculitis perhaps mediated by immune complexes although immunofluorescent studies have not confirmed this. These negative findings agree with results obtained in guinea pigs with induced hypersensitivity pneumonitis (22) but are in contrast to studies made upon human and bovine lung obtained from field cases (8, 19, 20). The presence of lymphocytes within inflammatory reactions in lung of calves in the present experiments, especially those in perivascular locations, may confirm delayed hypersensitivity which is suggested both by 24-28 hour respiratory response and by macrophage migration inhibition test results (21). While immunological and clinical indices of delayed hypersensitivity were most marked following exposures 3, 4 and 5, they were not accompanied by a remarkable histological evidence of delayed hypersensitivity in the corresponding lung biopsy. This may be explained by the extremely small sample obtained in the biopsy trephine with resultant probability of failure to observe typical changes.

The ease of induction of pulmonary hypersensitivity in calves is clearly demonstrated by the reactions obtained following few exposures to a very low concentration of *M. faeni* antigen. The clinical and histopathological response to such exposures suggests that the frequent occurrence of heavy environmental contamination with antigens derived from moldy feed has very high potential for induction of pulmonary disease in cattle and man.

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**REFERENCES**