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Pulmonary Multinucleate Giant Cells in Dermatosis Vegetans in Swine: Light microscopic and immunohistochemical investigations

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Evensen, Ø. and B. Bratberg: Pulmonary multinucleate giant cells in dermatosis vegetans in swine: Light microscopic and immunohistochemical investigations. Acta vet. scand. 1987, 28, 429-433. – Five pigs with dermatosis vegetans (DV), aged 1-28 days, were examined with the purpose of describing the pulmonary changes and to characterize the pulmonary multinucleate giant cells (MGC) and their possible cytogenesis.

No pulmonary changes were present at birth. From 7 days of age, lung changes were characterized by proliferation of alveolar epithelial cells and formation of MGCs. Immunostaining for cytokeratin by a peroxydase-streptavidin method gave a positive reaction in MGCs, bronchial, bronchiolar and alveolar epithelium. MGCs seemed to be formed in the course of alveolar epithelial proliferations, and type-II pneumocytes were proposed as possible precursors.

lungs; syncytia formations; cytokeratins; type-II pneumocytes.

Introduction

Dermatosis vegetans (DV) in pigs is a lethal, hereditary disease due to a recessive gene present in the homozygous state (*Percy & Hulland* 1968). The disease is characterized by skin and hoof lesions and pulmonary multinucleate giant cell (MGC) formations. The presence of MGCs has been regarded as the most reliable diagnostic feature of the disease (*Flatla et al.* 1961), and the lesions were referred to as a MGC pneumonitis by *Done et al.* (1967). *Percy & Hulland* (1968) described the formation of MGCs as associated with interstitial pneumonia or bronchopneumonia, developing as an integral part of the pulmonary inflammatory process. The authors concluded that MGCs were of mesenchymal origin and considered the alveolar macrophage as the most likely precursor.

The purpose of the present investigations is to describe the pulmonary changes and characterize MGCs with respect to morphology and possible cellular origin.

Materials and methods

Lung samples were obtained from 4 pigs out of a litter of 13 and 1 pig from a litter of 7, all with parents heterozygous for DV. Lung specimens from cranial and main lobes on both sides and bronchial and mediastinal lymph nodes were collected under deep pentobarbithal anaesthesia.

The specimens were fixed by immersion in 10% neutral buffered formalin, processed routinely and embedded in paraffin, sectio-

ned at 5-6 µm and stained with hematoxylin and eosin (HE) and van Gieson (vG).

Analogous specimens for semithin examination were fixed by immersion in Karnowsky with 0.2 mol/l cacodylate buffer, rinsed in the same buffer, postfixed in osmium tetroxide dehydrated in alcohol series, embedded in Epon LX 112, sectioned on Ultrotome Nova, LKB at 1 µm and stained with toluidine blue (TB).

All sections were examined in a Zeiss light microscope.

Specimens for immunohistochemistry were fixed in 10% neutral buffered formalin and embedded in paraffin. Deparaffinized sections were treated for 30 min. with 0.3% hydrogen peroxide in methanol to block endogenous peroxydase. After hydration, sections were incubated for 20 min. with 0.1% trypsin in 1% calcium chloride with pH adjusted to 7.8 by sodium hydroxide and then treated with normal goat serum (1:20) and foetal calf serum (FCS) (1:20) to block unspecific staining. Excess reagent was blotted from the slides before incubation with specific primary antiserum (1:250), antikeratin (Miles Scientific, code no. 65-792) for 30 min. Then the specimens were incubated with a 1:300 dilution of biotinlabelled secondary antibody for 30 min. and peroxydase-streptavidin reagent (1:300) (Amersham code no. RPN 1231) for 30 min. Tisusses were stained for 10 min. with 0.05% 3,3 diaminobenzidine tetrahydrochloride containing 0.01% hydrogen peroxide, counterstained for 2 min. in 10% hematoxylin and mounted in Difco aqueous mounting medium.

Normal guinea-pig serum was used as non-immune serum.

All dilutions were made with 3% FCS in PBS buffer as diluent, and all incubations were carried out at room temperature.

Results

Gross changes

Skin and hoof lesions were found as shown in Table 1.

There were no gross pulmonary changes indicating a pneumonia in the pigs examined.

Microscopic changes

At 1 day of age (Fig. 1a), lung samples showed normal well expanded lung tissues. Interalveolar septae were thin and slender and lined by low epithelium (Fig. 1b).

At 7 days of age, interalveolar septae were markedly thickened (Fig. 2a). Alveolar epithelial proliferation, characterized by cuboidal cells lining the alveolar septae, was appa-

Table 1. Experimental data.

Pig no.	Age when killed (days)	Hoof lesions	Skin lesions
1(1)*	1	+	-
2(1)	7	+	+
3(2)	14	+	+
4(1)	17	+	+
5(1)	28	+	+

* = litter no.

Figures 1-4. Lung samples from pigs with dermatosis vegetans.

Figure 1 a. 1 day old. Normal lung tissue with smooth septae and little branhing. Hematoxylin and eosin (HE). × 80.

Figure 1 b. 1 day old. Alveolar septae. Toluidine blue (TB). × 500.

Figure 2a. 7 days old. Thickened alveolar septae, atelectasis and emphysema. HE. × 80.

Figure 2b. 7 days old. Alveolar septum, detail. Proliferation of alveolar epithelial cells (arrows). TB. × 500.

Figure 2c. 7 days old. MGCs located adjacent to the alveolar septum (arrows). TB. × 320.

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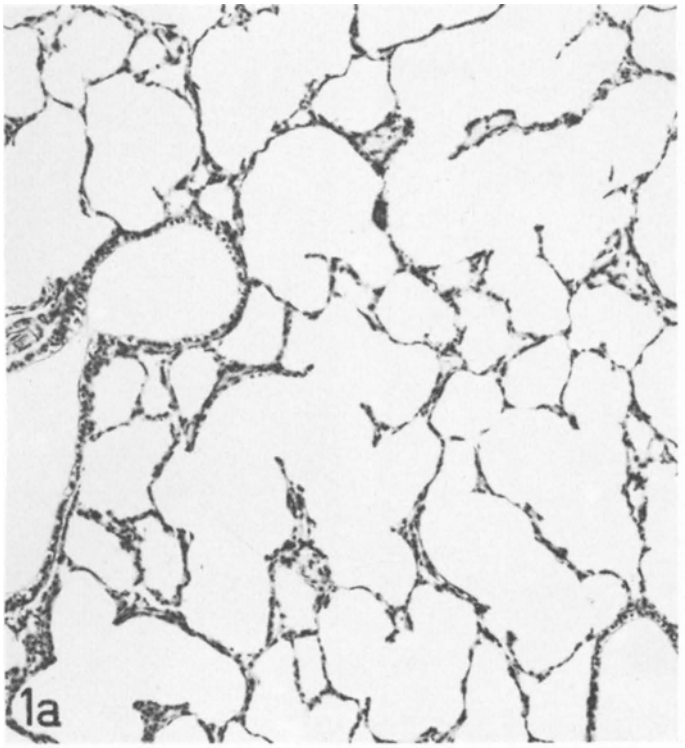

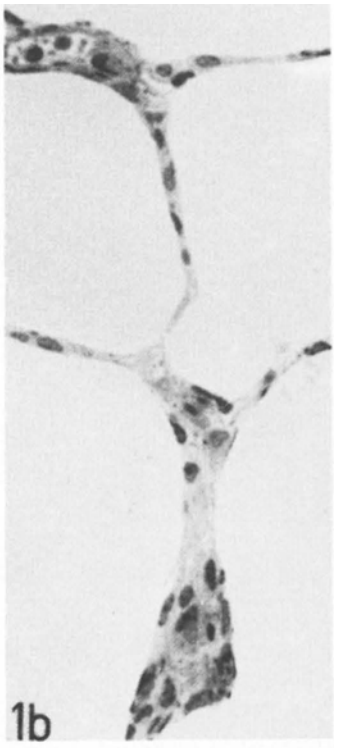

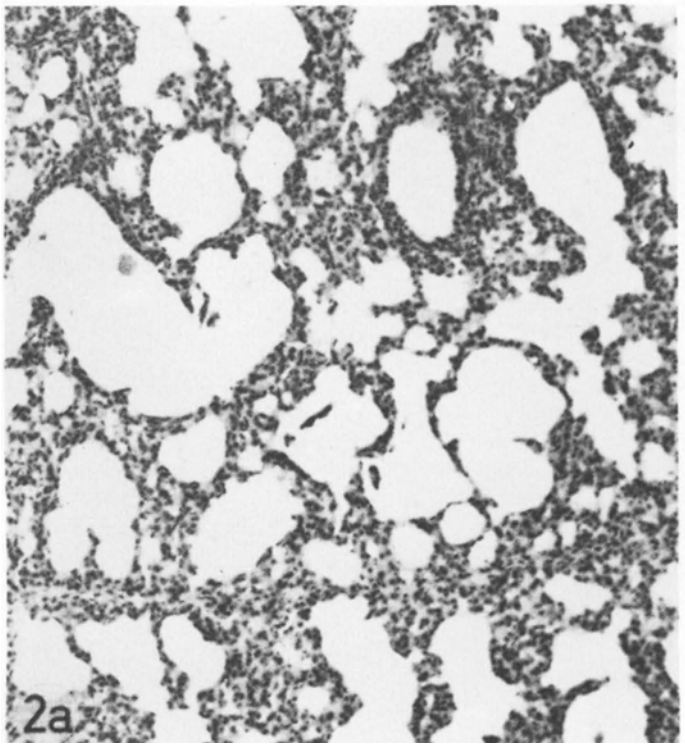

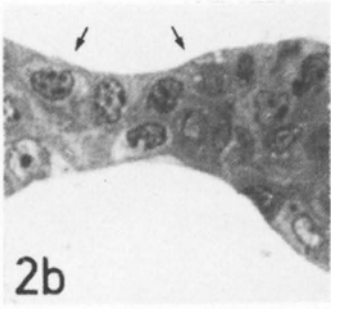

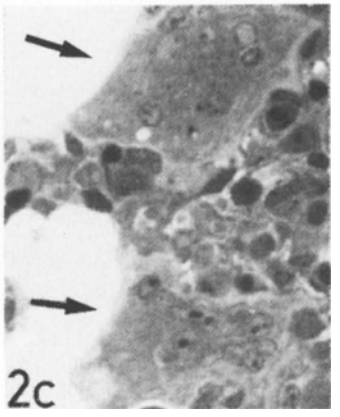

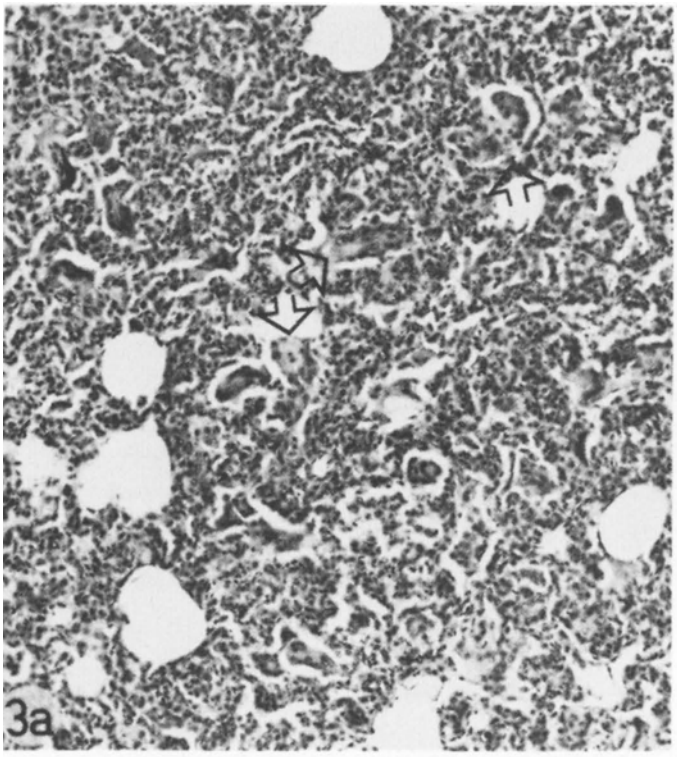

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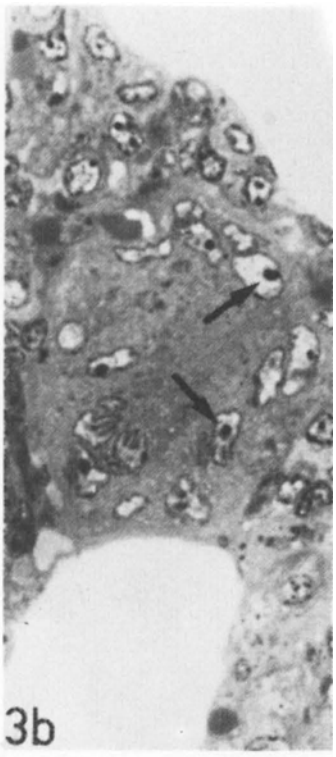

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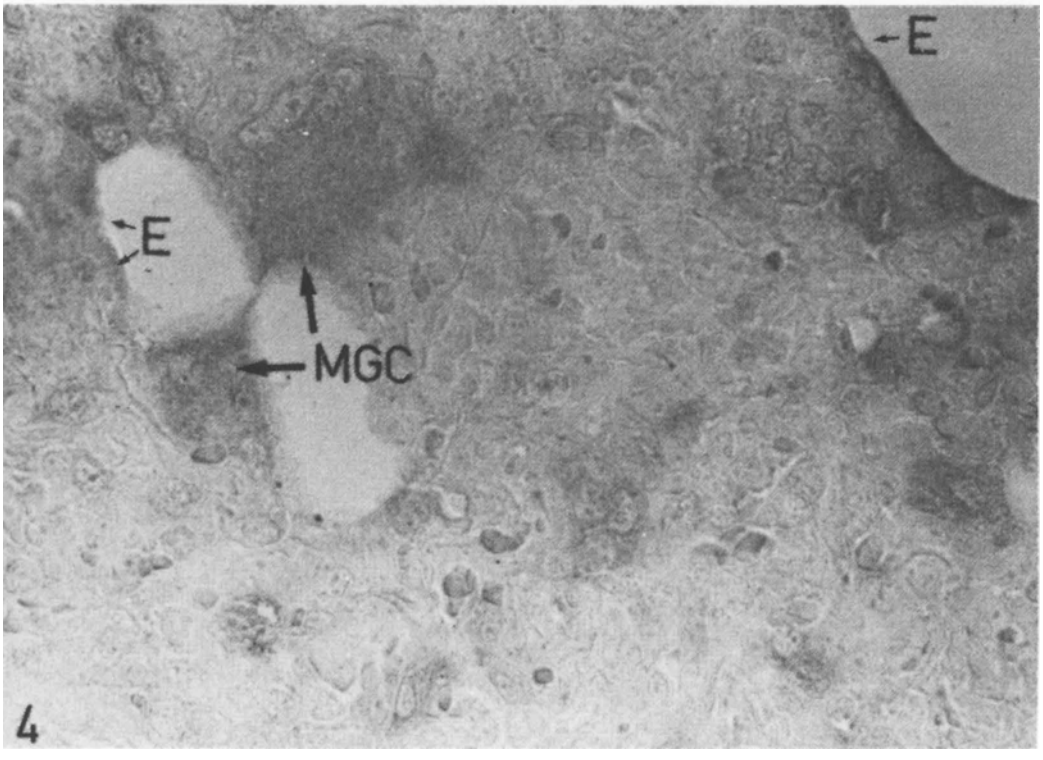

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rent (Fig. 2b). Lung tissue was atelectic in large areas. MGCs were found at this age (Fig. 2c).

At 14 and 17 days of age, lung tissues were markedly atelectic (Fig. 3a). The thickening of alveolar septae and alveolar epithelial proliferation was even more pronounced at this age. MGCs were numerous (Fig. 3a).

At 28 days of age, lesions were similar to those described at 14 and 17 days of age with numerous MGCs formations.

MGCs were found from 7 days of age (Fig. 2c). They were pleomorphic varying in size up to 60 µm in diameter. The number and localization of nuclei varied between cells (Fig. 2c and 3b). Prominent nucleoli could easily be demonstrated (Fig. 3b). MGCs were found both free in the alveolar lumen and in the alveolar wall (Fig. 3b). Mitotic figures were not observed within MGCs.

Immunostaining for cytokeratin gave positive reaction in MGCs (Fig. 4). Incubation with non-immuneserum or secondary antibodies gave no specific staining of MGCs. A slight background staining in interlobular connective tissue was observed. Bronchial and alveolar epithelium showed a positive staining for cytokeratin.

It was not possible to demonstrate MGCs within bronchial or mediastinal lymph nodes.

Discussion

The present investigations demonstrate proliferation of alveolar epithelial cells and formation of pulmonary multinucleate giant cells. Positive immunostaining for cytokeratin gives substantial evidence for an epithelial origin of pulmonary MGCs, and the type-II pneumocyte is proposed as a possible precursor.

Lung parenchyma in newborn pigs is characterized by smooth and straight septal walls with occasional ramifications. The present study showed large airspaces at the alveolar level in the newborn pig, in accordance with previous descriptions (*Winkler & Cheville* 1984).

The principal pulmonary lesions observed in pigs with DV were, according to *Jericho* (1974), characterized by proliferation of alveolar septal cells. The proliferative responses of type-II pneumocytes have been used as an indirect means to quantify alveolar damage (*Evans et al.* 1978). However, there are observations that describe increased proliferation without any morphologic evidence of cellular injury to alveolar epithelium (*Barry et al.* 1983), but under such circumstances proliferation of type-II pneumocytes is correlated to the influx of inflammatory cells (*Shami et al.* 1986).

According to *Done et al.* (1967) and *Percy & Hulland* (1968), pigs with DV die with an interstitial pneumonia and/or a bronchopneumonia.

The present results are in contrast to these findings. Proliferation of alveolar epithelial cells seems to evolve independently of an inflammatory response and without obvious signs of epithelial damage. The mechanisms responsible for these changes are unknown. Factors controlling the rate and extent of type-II pneumocyte proliferation may be influenced through a genetic disorder.

Figure 3a. 17 days old. Pronounced atelectasis and numerous MGCs (arrows). HE. × 80.

Figure 3b. 17 days old. MGCs located in the alveolar septum. Several nuclei with various forms and prominent nucleoli (arrows). TB. × 500.

Figure 4. 14 days old. Immunoperoxydase staining. Positive reaction for cytokeratin in MGCc and alveolar epithelium (E). Counterstained with 10% hematoxylin. × 400.

MGCs were observed from 7 days of age in agreement with the results of *Percy & Hulland* (1968). The same authors and *Done et al.* (1967) considered the MGCs to be of mesenchymal origin, and the alveolar vesiculate septal cell, which they considered as the precursor for alveolar macrophages, was considered as the most likely precursor.

The septal cell, large vesiculate alveolar septal cell or large vesicular cell described by *Percy & Hulland* (1968), may according to current concepts be considered synonomous with type-II pneumocytes.

Syncytia are according to *Papadimitriou & Walters* (1979) multinucleate giant cells formed through fusion of mononuclear precursors. Syncytia of macrophage origin are usually associated with inflammations and especially granulomatous inflammations. Pulmonary syncytia are also seen in parainfluenza-3 (*Bryson et al.* 1979) and respiratory syncytial virus infections in cattle (*Bryson et al.* 1983).

Thus, the prerequisites for macrophage-derived syncytia are not fulfilled either in our material or in previous investigations and the pulmonary MGCs in DV are therefore presumably not of macrophage origin.

Components of the cytoskeleton of the »intermediate« size, especially the keratins, are characteristics of epithelial cells (*Moll et al.* 1982). There are, however, little information available regarding the keratin content and diversity of alveolar epithelial cells. *Schlegel et al.* (1980) concluded that normal alveolar epithelial cells did not contain keratins. According to recent reports (*Blobel et al.* 1984; *Vogel & Gown* 1984) alveolar epithelium reacts with some antibodies. *Woodcock-Mitchell et al.* (1986) have also characterized a monoclonal antibody that reacts with type-II like cells in rats after lung injury but not with normal type-II pneumocytes. They propose that keratins in type - II pneumocytes undergo a conformational change after injury and become reactive with the monoclonal antibody. The polyclonal antibodies used in the present study were raised against keratin isolated from fetal bovine hooves. The positive immunostaining of alveolar epithelial cells corresponds with the observed proliferation of alveolar epithelial cells. The positive staining of MGCs gives substantial evidence for an epithelial origin. On the basis of concurrent proliferation of alveolar epithelial cells and localization of MGCs in the alveoli, it is also likely that the type-II pneumocyte is a precursor. Electron microscopic examinations in progress will provide further evidence.

Percy & Hulland (1968) suggested an evolution of MGCs in DV through nuclear division without concurrent cytoplasmic division. Recent studies indicate that the most probable mechanism in the formation of syncytia is through fusion of mononuclear precursors (*Papadimitriou & Walters* 1979).

Percy & Hulland (1968) argued that the close proximity of several nuclei indicated repetitive nuclear divisions without concurrent cytoplasmic divisions. This characteristic is also observed in foreign body inflammatory reactions with the formation of syncytia and seems to be a typical feature of recently formed MGCs (*Mariano & Spector* 1974).

It may be concluded from the present studies that proliferation of alveolar epithelial cells and formation of MGCs of possible epithelial origin are the key pulmonary changes in DV.

Acknowledgment

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Sammendrag

Pulmonære multinukleære kjempeceller ved dermatosis vegetans hos gris. Lysmikroskopiske og immunhistokjemiske studier.

Fem griser i alderen 1 til 28 dager med lidelsen dermatosis vegetans (DV) ble undersøkt med henblikk på å beskrive lungeforandringer og spesielt for å gi en morfologisk karakterisering av pulmonære multinukleære kjempeceller (PMK) og deres mulige cytogenese.

Det forelå ingen lungeforandringer ved fødsel. Fra 7 dagers alder var lungeforandringene karakterisert ved proliferasjon av alveole-epitelceller og PMK-dannelser. Ved immunhistokjemiske studier basert på en peroksydase-streptavidin metode ble det påvist positiv reaksjon i PMK, bronkie-, bronkiole- og proliferert alveole epitel.

PMK syntes å dannes i forløpet av alveolære proliferasjoner og type-II pneumocytter ansees som en mulig cellulær opprinnelse.

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