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Autor: González, L. / Cuervo, L.A. / Juste, R.A.

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PATHOLOGICAL AND EPIDEMIOLOGIC ASPECTS OF THE CONCURRENCE OF MAEDI AND SHEEP PULMONARY ADENOMATOSIS

L. González, L. A. Cuervo, R. A. Juste, I. Idígoras

The simultaneous occurrence of maedi/visna (MV) and sheep pulmonary adenomatosis (SPA) has been frequently recorded in sheep flocks in several countries (Pálsson, 1976; Markson and other, 1983; Snyder and others, 1983, Houwers and Terpstra, 1984). In some of these reports, specific reference has been made to the exceptionally high seroprevalence of MV virus (MVV) infection and to the severe losses derived from MV in such flocks. These findings have been experimentally shown to be due to an enhanced and more effective lateral dissemination of MVV by animals suffering from SPA (Dawson and others, 1985) simultaneously.

The present paper provides further evidence of the epidemiologic importance of SPA in the spread of MV virus. The pathological aspects of the concurrence of these virus diseases and the reasons that explain the relevance of their simultaneous appearance are discussed. The results here presented come from a large scale study on chronic respiratory diseases carried out in the Basque Country (Spain) during 1982-1989.

Material and methods

Epidemiologic survey: Serological analyses were performed on 136 flocks that comprised a sheep population of 28.065 animals. From each flock 30 sheep older than one year were selected at random and bled. A total of 4.080 serum samples were tested for antibodies to MVV, using the agar-gel immunodiffusion test (AGIDT) described by Winward and others (1979). The antigen was prepared from sheep choroid plexus cell cultures infected with the WLC-1 strain of ovine progressive pneumonia virus (OPPV).

Pathological studies (see below) confirmed the presence of SPA in 29 flocks, the serological results of which were compared with those of the remaining 107 by means of an analysis of variance.

Studies on individual sheep: 133 animals showing clinical signs of chronic respiratory disease were subjected to necropsy and laboratory examinations in order to identify the specific pneumonias that had caused the affections. These animals belonged to 84 flocks of the 136 mentioned above.

Laboratory examinations included routine gross pathology and histopathology of the lungs and serological tests for MVV antibodies (AGIDT). In order to evaluate the concurrence of lesions of maedi and SPA, pieces of lung from tumour-like nodules/areas, from non-consolidated parenchyma and from bordering areas between these two were subjected to histopathological examination.

Results

Epidemiology: In the 29 sheep flocks in which the presence of SPA was confirmed (SPA+), the average seroprevalence of MVV infection was 68.0%, whereas in the remaining 107 flocks (SPA?) the seroprevalence was 47.3%. These values were found to be significantly different (p. <0.0001).

Figure 1 shows the distribution of flocks of both groups according to the level of MVV infection. None of the SPA+ flocks showed lower values than 30%, whereas in 75% of them the seroprevalence of MVV infection was higher than 60%. In contrast, in 15% of the

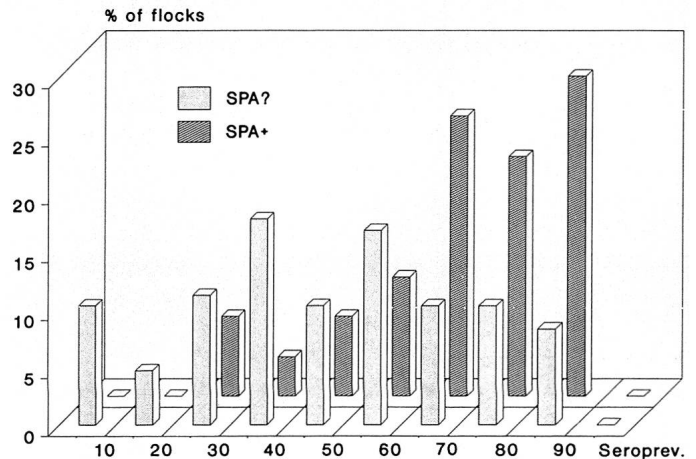


Fig. 1: Distribution of SPA? and SPA+ flocks according to their seroprevalence of MVV infection.

SPA? flocks the prevalence of this infection was lower than 30% and only 29% had higher values than 60%.

Individual sheep examinations: According to the features and extension of the pulmonary lesions, 85 animals were diagnosed as having maedi (interstitial mononuclear pneumonia accompanied by lymphoid follicular hyperplasia and smooth muscle hypertrophy) and 33 as having SPA (bronchioalveolar adenocarcinoma accompanied by desquamative pneumonia). The other 15 cases were identified as verminous or bacterial bronchopneumonias.

Four of the former 85 showed discrete lesions of SPA. From the 33 SPA affected animals, 2 also showed characteristic though moderate lesions of maedi in the non-consolidated lung parenchyma and another 3 exhibited lesions compatible but inconclusive of maedi. To summarize, concurrence of lesions of maedi and SPA, when the latter was the cause of the respiratory affection was a rare event (2/33 = 6.1% to 5/33 = 15.5%).

Serological examinations indicated that 93% (79/85) of the maedi affected animals had antibodies to MVV. 24 of the 33 SPA diagnosed sheep (72%) proved to be also infected by MV virus, according to the results of the AGIDT.

Discussion

The epidemiologic results confirm the importance of the occurrence of SPA in the seroprevalence of MV virus infection in sheep flocks. We also must consider that in an indeterminate number of our SPA? flocks, probably in those with high MV seroprevalences, SPA is certainly present, although it has not been detected so far. A hypothetical comparison between SPA affected and SPA free flocks

would presumably have shown greater differences in the seroprevalence of MV virus infection.

To explain the relevant role of this transmissible tumour, the coexistence of maedi and SPA in individual sheep has to be a rather frequent happening. Our results indicate that the concurrence of lesions of both diseases is an unusual finding that can not justify the epidemiologic results.

Nevertheless, we found a high proportion of SPA affected animals to be also infected by MV virus. As the affinity of this virus for the alveolar macrophages has already been demonstrated (Narayan and others, 1982), it is likely that these animals harbour MVV in those target cells, which are abundant and characteristic in SPA lungs. This possibility, which is also taken into account by other authors (Dawson and others, 1985), would explain the epidemiologic role of SPA in the lateral transmission of MV.

We conclude that the favourable conditions for the respiratory transmission of maedi/visna in sheep flocks depend upon the presence of animals that, being MVV infected, show lesions and clinical signs of SPA. In those animals there would be no need of development of lesions and symptoms of maedi, a fact that seems to be important for the lateral spread of MV, when it occurs as single infection (Palsson, 1976).

Institut of Animal Neurology, University of Bern, Switzerland

TOXIC EFFECT OF REACTIVE OXYGEN SPECIES ON OLIGODENDROCYTES

C. Griot, M. Vandeveld, A. Richard, E. Peterhans, R. Stocker

The mechanism underlying demyelination in the CNS of dogs suffering from canine distemper (CD), an animal model for multiple sclerosis, is still unclear. Macrophages and their secretory products have been suggested to be partly responsible for the severe necrosis observed in the white matter of animals with CD that develop chronic inflammatory lesions. We have shown that anti-CD virus antibodies binding to CD virus-infected glial cells are capable to stimulate brain macrophages *in vitro* leading to the release of reactive oxygen species (ROS), potentially harmful products (Bürge et al., 1989, Griot et al., 1989). It is likely that these events also occur *in vivo* since CD virus-infected glial cells, macrophages and antiviral antibodies are present in close proximity in chronic inflammatory lesions in CD. Activated macrophages generate superoxide (O_2^-) and hydrogen peroxide (H_2O_2) as primary reduction products. In the presence of transition metals such as Fe^{++} , O_2^- and H_2O_2 can give rise to highly toxic hydroxyl radicals ($^{\circ}OH$) which are known to cause considerable tissue damage through reaction with DNA, protein and membrane lipids (Halliwell and Gutteridge, 1989). Since the above described tissue destruction is predominantly seen in the white matter, we examined whether myelin, or oligodendrocytes (the myelin producing cells in the CNS) are particularly susceptible to ROS.

Therefore, we exposed primary cell cultures prepared from neonatal dog cerebella to xanthine/xanthine oxidase (X/XO), a well known O_2^- producing system (Griot et al. in press). Oligodendrocytes, astrocytes and brain macrophages (the main celltypes present in these cultures) were visualized using immunocytochemical methods and examined using a light microscope.

In maedi affected animals, a correlation between the rate of replication of MVV in pulmonary macrophages and the presence of characteristic lesions has been described, so that the expression of viral antigens in the surface of these cells starts the inflammatory response (Lairmore and others, 1988). According to our results, this does not seem to happen when the infected macrophages are those of SPA. There, MVV appears to replicate without eliciting any clear or constant lymphocytic response, but the mechanisms to explain this are still obscure.

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This treatment resulted in a specific time-dependent degeneration and loss of oligodendrocytes whereas the morphological appearance of astrocytes and macrophages was unchanged (Figure). Further, an

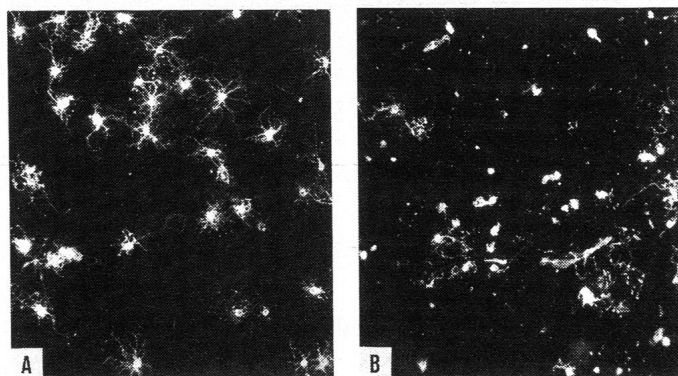


Fig. 1: Morphological appearance of FITC-immunostained oligodendrocytes in normal (A) and X/XO-treated glial cell cultures (B) in which most of the oligodendrocytes show severe signs of degeneration. x100.

evaluation of the effect of several ROS scavengers and transition metal chelators suggests that a metal-dependent formation of $^{\circ}OH$ could be responsible for the observed damage (Table).

The exact biochemical mechanism of oligodendroglial degeneration mediated by ROS needs still further investigation which may ultima-