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PERSISTENCE OF PNEUMONIA VIRUS OF MICE AND SENDAI VIRUS IN GERM-FREE (*nu/nu*) MICE

P. CARTHEW AND S. SPARROW

From the Medical Research Council Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF

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Summary.—The pathogenicity and persistence of pneumonia virus of mice (PVM) and Sendai virus has been studied using germ-free *nu/nu* mice. PVM was found to infect cells of the bronchial epithelium (and the alveolar wall) of the lungs of germ-free *nu/nu* mice using the immunoperoxidase technique. The virus was located in the bronchial epithelium for 11 days before elimination, but persisted in the alveolar wall for the duration of the experiment (20 days). After Day 10 a humoral antibody response to PVM was observed which persisted, although at a low level (1 in 40), by haemagglutination-inhibition (HI) testing.

Sendai virus in *nu/nu* mice also infected cells of the bronchial epithelium and this persisted for the duration of the experiment (27 days). The persistence of virus in the bronchial epithelium in relation to lack of humoral antibody is discussed with reference to local secretory antibody production, especially since this does not occur with PVM.

THE PATHOGENICITY of Sendai virus has been studied by several authors (Robinson, Cureton and Heath, 1968; Mimms and Murphy, 1973; Carthew and Sparrow, 1980). The main findings have been that virus replicates in the bronchial epithelium for 7–8 days before its elimination and the development of circulating humoral antibody. Similarly, PVM has been found to infect the bronchial epithelium and alveolar walls of mouse lungs for 8 days before its elimination and the development of humoral antibody (Carthew and Sparrow, 1980; Tennant, Parker and Ward, 1965). However, no circulating antibody has been demonstrated for *nu/nu* mice infected with Sendai virus (Ward *et al.*, 1976; Ueda *et al.*, 1977) and the persistence of Sendai virus in the lungs of *nu/nus* has not been studied. Similarly, neither the persistence of PVM in *nu/nu* mice nor their antibody response to it has been studied. In view of the importance of this athymic strain of mouse to tumour studies and its long-term maintenance in colonies, this particular study has been undertaken to determine the possibility of persistence and the immune response of these two viruses in *nu/nu* mice.

MATERIALS AND METHODS

Six- to 8-week-old, germ-free mice were used for the study. Infection with PVM was achieved by intranasal inoculation with 0·025 ml of tissue culture fluid containing 10^5 TCD_{50} of PVM. Infection with Sendai virus was with a dose of 0·025 ml containing 10^5 EID_{50}.

Mice were maintained in filter boxes after inoculation and killed at intervals. Blood was taken and examined for HI antibodies to PVM over the duration of the experiment (Ward *et al.*, 1976). Lungs were removed and processed by the method of Sainte Marie (1962) and examined by immunoperoxidase staining for Sendai virus and PVM as described previously (Carthew and Sparrow, 1980). Haematoxylin and eosin sections were also examined.

RESULTS

Sendai-virus-infected mice

The earliest changes seen in the lung with Sendai virus were at 2 days after infection. The cytoplasm of the bronchial

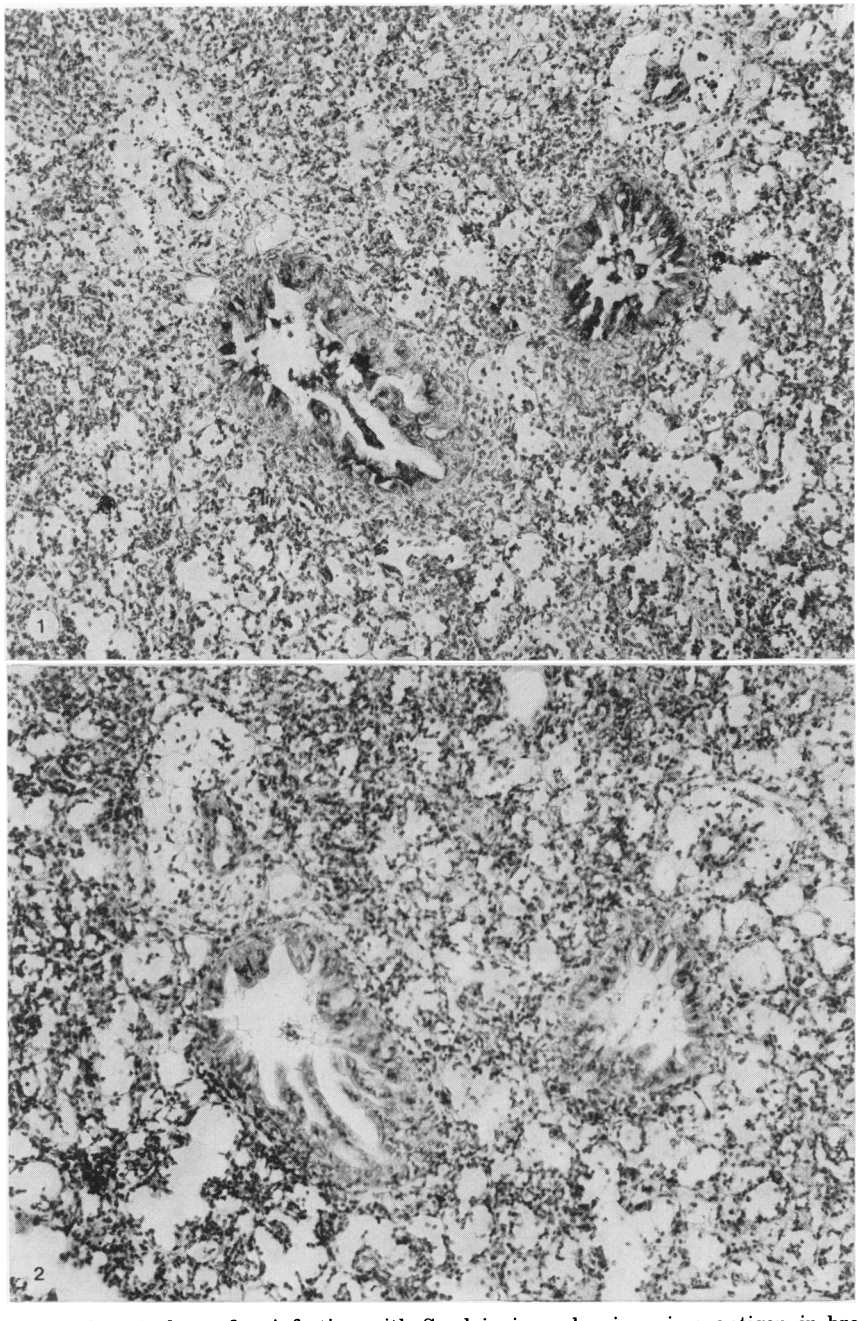

FIG. 1.—Mouse lung 7 days after infection with Sendai virus, showing virus antigen in bronchial epithelium. Peroxidase stain, counterstained with haematoxylin. × 120.

FIG. 2.—Similar section to Fig. 1, but stained with haematoxylin and eosin to show peribronchial lymphocyte accumulation. × 120.

epithelium was hyperchromatic and some cytoplasmic débris were being shed into the bronchial lumen. There was also some sticking of polymorphs to arterial walls and migration of these cells through the walls and a slight accumulation of mononuclear cells in the peribronchial regions. This latter feature became much more marked in subsequent days so that substantial areas of consolidation appeared around the bronchi and bronchioles. There was also stripping of the bronchial epithelium and the formation of small plugs of cellular débris (mostly epithelial cells and polymorphs, but also some macrophages at later stages) in the bronchial lumen. There was only slight hyperplasia of the bronchial epithelium. There was marked peroxidase staining of the bronchial epithelium from Day 3 until 31 days after infection (Fig. 1), when the last animal was killed, although there were 3 days (Days 6–8) when no epithelial staining was observed. During this period (Day 7) a number of alveolar cells and a localized aggregation of macrophages in the same area were stained. The only staining observed was alveolar macrophages from Day 7 to Day 24 after infection.

PVM-infected mice

With PVM the changes in the bronchial epithelium were less marked and there was no peribronchial mononuclear activity. At 2 days after infection, however, there was thickening of the alveolar septa and some oedema. These lesions rapidly progressed with cellular infiltration of the alveolar tissue until at Day 4 there were large areas of consolidation. The alveolar changes were seen until the last animal was killed on Day 20. There was cellular infiltration by some mononuclear cells and large numbers of macrophages.

Peroxidase staining of tissue showed viral replication in the bronchial epithelium and alveolar cells. Maximum staining in the bronchial epithelium occurred at Day 5 after infection and only very small amounts were seen at Day 11. No bronchial staining was seen after this time. Alveolar cells and alveolar macrophages, however, continued to be stained until 20 days after infection.

The reaction of the *nu/nu* mouse lung to the 2 viruses is similar but not identical to that of euthymic animals (Carthew and Sparrow, 1980). In particular, the extreme bronchial hyperplasia and the widespread formation of bronchial plugs leading to localized collapse of lung tissue were not prominent features. The peribronchial and perivascular mononuclear accumulation with Sendai virus was more noticeable in the *nu/nu* mice (Fig. 2) and the alveolar thickening seen with PVM was more rapid with *nu/nu* mice. Immunoperoxidase staining of sections of PVM-infected lungs with anti-mouse IgG showed no localized production of IgG in the lung. Humoral antibody response to PVM infection was absent in sera tested from Day 0 to Day 10 but from Day 11 to Day 20 HI titres of 1 in 40 were found in all sera examined.

DISCUSSION

It is interesting to note that while Sendai virus persisted in the bronchial epithelium of *nu/nu*s for at least 21 days (and probably for the duration of life) in the absence of humoral antibody response, mice infected with PVM tended to eliminate the virus from the bronchial epithelium after 5 days and virus persisted only in the alveolar wall. This would be consistent with the development of measurable humoral antibody response in *nu/nu*s to PVM which, although low (1 in 40), is very significant.

The absence of local IgG production in the lung suggests that secretory antibody of the IgA or IgM class is being locally produced at the bronchial epithelium (but not in the alveolar wall) to neutralize the infectious virus. This would be consistent with the postulate of Blandford (1975) that different classes of antibody can be produced at different secretory surfaces in the lung to combat particular virus infections. It seems that this model system

could be valuable in the study of the mechanism of immune-deficiency virus disorders.

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