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Interstitial Pneumonia Induced by a Plaque-Type Variant of Mouse Adenovirus[1] (41179)

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Abstract. A plaque-type variant of mouse adenovirus, strain FL was isolated using L-cell monolayer cultures for the plaque assay. Intraperitoneal inoculation of this variant strain was observed to cause death accompanied by hemorrhagic lungs and interstitial pneumonia in adult C3H/HeN mice. Suckling mice developed patchy interstitial pneumonia after intranasal inoculation. Lethal disease was associated with infectivity (LD_{50} = 5.0 × 10^6 PFU) and the virus was detected in the lungs by electron microscopy. This plaque-type variant may provide a useful model that heretofore was unavailable for the study of adenovirus pathogenesis in pulmonary disease.

A paucity of information regarding the pathogenesis of human adenovirus respiratory infections exists due to the marked species specificity of adenoviruses and a lack of a suitable laboratory animal model. In regard to natural adenovirus infection of lower animals, certain strains of canine, porcine, bovine, simian, and equine adenoviruses are associated with respiratory disease in their respective hosts (1–10). However, the size and cost of these species impeded their development as animal models for the study of adenovirus respiratory infection.

Type 1 mouse adenovirus (MAd1), a member of the Adenoviridae family (11–15), is not considered a candidate virus for the study of respiratory infection, since MAd1 is generally associated with a systemic disease in mice (16–19). Respiratory disease or pathologic changes in lung tissues following MAd1 infection of mice have not been reported. This report describes the isolation of an MAd1 plaque-type strain that induces respiratory disease in the form of a classic adenovirus interstitial pneumonia.

Materials and Methods. *Virus.* MAd1 (American Type Culture Collection: Rockville, Md.) was propagated on monolayer cultures of L cells (NCTC Clone 929, American Type Culture Collection) maintained with Eagle's MEM supplemented with 5% fetal calf serum, streptomycin (0.1 mg/ml), and penicillin (100 units/ml). Virus titrations were performed by using a plaque assay (20) with L-cell monolayer cultures in 60-mm dishes. Infectivity was expressed as plaque-forming units (PFU). The median lethal dose of virus was calculated by the method of Reed and Muench (21). Isolation of MAd1 plaque types was obtained by inserting a bacteriological inoculating needle through the agar overlay medium to the center of a well-isolated plaque. Culture medium (1 ml) was then inoculated with the needle and plaques were developed directly from the inoculated medium. This technique resulted in 5 to 50 plaques per dish.

Animals and housing. Male C3H/HeN (*nu*/+) mice were bred and housed as described previously (22). Male C3H/HeJ mice were supplied by Dr. John Hackney (Department of Pharmacology and Therapeutics, College of Medicine, University of South Florida, Tampa, Fla.) and were housed under conventional conditions.

Light and electron microscopy. Fixation of the lungs removed at necropsy was car-

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ried out by either immersion of the lungs in a fixative (2% *p*-formaldehyde–3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3), or perfusion of the lungs by infusion of fixative through the right ventricle. Six-micrometer paraffin-embedded sections were stained with hematoxylin–eosin and ultrathin epon-embedded sections (60 to 70 nm) were stained with uranyl acetate and lead citrate as described previously (22).

Neutralization tests. Antiserum titers were obtained using a microtiter system. All sera to be tested were diluted fivefold in saline and incubated at 56° for 30 min. Twofold dilutions of the sera were incubated at 37° for 3 hr with 100 median tissue culture infective doses ($TCID_{50}$) of virus challenge. The virus–serum reactions were added to triplicate L-cell cultures in flat-bottom microtest plates and cytopathic effects (CPE) were recorded after 10 days. Control serum consisted of pooled serums obtained from random bleeding of the C3H/HeN colony. Reference mouse adenovirus antiserum was obtained from Dr. Jack Gruber (National Cancer Institute, Bethesda, Md.). Antiserum against the plaque-type variant strain was obtained from C3H/HeN mice 24 days after intraperitoneal inoculation with 3.2×10^6 PFU.

Results. *Isolation of a plaque-type strain of MAd1.* Development of mouse adenovirus plaques in L-cell monolayer cultures revealed a variety of plaque morphologies upon neutral red staining. Plaque diameters ranged from 0.3 to 3.4 mm. One distinct plaque morphology appeared as a minor component of the plaque population. This plaque-type morphology exhibited a relatively large, variable size and a defined border when compared to the remaining plaque population. Microscopic examination of these plaques indicated that defined border plaques resulted from essentially 100% cell death within the plaque. Plaque specimens were propagated and the cultures that exhibited defined plaques exclusively were used for three subsequent cycles of plaque isolation. High-titer stock virus was prepared from a representative plaque obtained on the third cycle of plaque purification. The defined border plaque type appeared to be a stable trait, and this strain was designated type 1 mouse adenovirus, plaque type 4 (MAd1*pt*4).

*Dose response of MAd1*pt*4 and MAd1 in adult mice.* Inoculation (ip) of adult C3H/HeN mice with high doses of MAd1*pt*4 resulted in clinical disease (marked physical inactivity, hunched back, and puffed fur) and death (Table I). Death occurred 3–5 days after infection. Lower doses of the virus resulted in decreased mortality and increased survival times. All mice exhibiting lethal disease died within 10 days and collapsed hemorrhagic lungs were observed on necropsy. Mice surviving 10 days of infection recovered apparent health.

Inoculation (ip) of adult C3H/HeJ mice with comparable high doses of the MAd1 parent strain also resulted in clinical disease and death (Table I). However, hemorrhagic lungs were not observed on necropsy. Death occurred 4–5 days after infection. A median lethal dose of 3.0×10^6 and 5.0×10^6 PFU was calculated for MAd1 and MAd1*pt*4, respectively. Although MAd1*pt*4 did not appear to be more virulent than the parent MAd1, the new plaque-type strain possessed a distinct pulmonary tropism.

*Histology of the MAd1*pt*4-induced pneumonia.* Extensive infiltration of erythro-

TABLE I. DOSE–RESPONSE OF MOUSE ADENOVIRUSES IN ADULT MICE

Strain	Inoculum (PFU)	Mortality (death/total)	Time of death (days)	Macroscopic lesions (No. observed)
MAd1*pt*4	1.0×10^8	15/15	3–5	15[a]
	1.0×10^7	8/10	6–10	8[a]
	1.0×10^6	1/10	9	1[a]
MAd1	1.0×10^7	18/18	4–5	0
	1.0×10^6	1/9	7	0

[a] Hemorrhagic lungs.

cytes and lymphocytes into the interstices was observed in hemorrhagic lungs obtained at necropsy of adult mice inoculated with 10^8 PFU (Fig. 1). Examination of bronchiole epithelium revealed a hyperplastic response and patchy necrosis. Plugs of sloughed epithelial cells were observed in terminal bronchioles. Mice that were inoculated ip with sublethal doses of virus (10^6 PFU) and sacrificed 4 days after infection exhibited interstitial pneumonia, but with less extensive involvement. In addition, intranasal inoculation of suckling C3H/HeN mice with MAd1*pt*4 resulted in a patchy interstitial pneumonia without obvious signs of clinical disease. Although the lungs appeared normal macroscopically, microscopic examination revealed patchy interstitial pneumonia in all inoculated mice 7 days after infection.

Association of replicating MAd1 pt*4 with the interstitial pneumonia.* Adult C3H/HeN mice were inoculated with high doses of MAd1*pt*4 (1×10^8 PFU). The animals were sacrificed by cervical dislocation just prior to the expected time of death (3.5 days after infection). The vascular beds of the lungs were fixed by perfusion of fixative through the right ventricle and sections for electron microscopy were prepared. Intranuclear adenovirus particles were observed in endothelial cells of the lung tissue (Fig. 2). Thus, replicating MAd1*pt*4 was associated with the pulmonary lesion.

Serologic confirmation of MAd1 pt*4 as a mouse adenovirus.* MAd1 and MAd1*pt*4 were closely related serologically. Reference mouse adenovirus antiserum (raised against serotype 1 mouse adenovirus) neutralized the CPE of both MAd1 and MAd1*pt*4 (Table II). In addition, reaction of reference MAd1 antiserum with high doses of MAd1*pt*4 (1×10^8 PFU) abrogated the lethal effects of infection (unpublished data). Antiserum raised against MAd1*pt*4 neutralized the CPE of both MAd1 and MAd1*pt*4 also.

Discussion. A variety of factors are involved in viral virulence and plaque formation. Consequently, it is difficult to correlate a given plaque characteristic with virulence in animal studies (23). However, the initial approach to isolation of MAd1 strains, which might exhibit enhanced virulence, was directed toward the isolation of relatively large plaques that could result possibly from a high degree of cell lethality and increased efficacy of replication in murine cells. Tentatively, MAd1*pt*4 fulfilled these criteria. The isolated plaque type was stable, *in vitro* and *in vivo*. Three consecutive plaque isolations exhibited the same morphology as the original plaque isolate and the plaque type remained unchanged after passage through mouse infection. MAd1*pt*4 did not appear to have a notable degree of increased virulence, as compared to MAd1. The median lethal doses of MAd1*pt*4 and the MAd1 parent strain (5×10^6 and 3×10^6 PFU, respectively) were similar, but MAd1*pt*4 possessed a distinct pulmonary tropism.

The histology of MAd1*pt*4-infected lungs mimicked human adenovirus respiratory disease (24). Development of the characteristic pulmonary lesions was not dependent on the route of inoculation, since both intraperitoneal and intranasal inoculation resulted in the interstitial pneumonia. The degree of pneumonia following intranasal inoculation appeared to be less severe; however it was difficult to evaluate quantitatively due to the inaccuracy of the inoculation method.

The question remains as to why MAd1 did not induce the pulmonary lesions when it apparently contained MAd1*pt*4 within its plaque population. One explanation could be that the number of MAd1*pt*4 virions within the MAd1 plaque population was insufficient to cause pulmonary disease. A second explanation could be that an interference phenomenon between MAd1*pt*4 and other plaque types within the MAd1 population existed. A third explanation could be that the selection process for MAd1*pt*4 might have isolated a newly derived mutant strain due to serendipity. Thus, the true size of the apparent MAd1*pt*4 population within MAd1 might be overestimated on the basis of plaque morphology alone. Serologic analysis of MAd1 and MAd1*pt*4 established that MAd1*pt*4 was closely related to MAd1, however, the serologic relatedness did not rule out the selection of a newly derived strain that had mutated in an

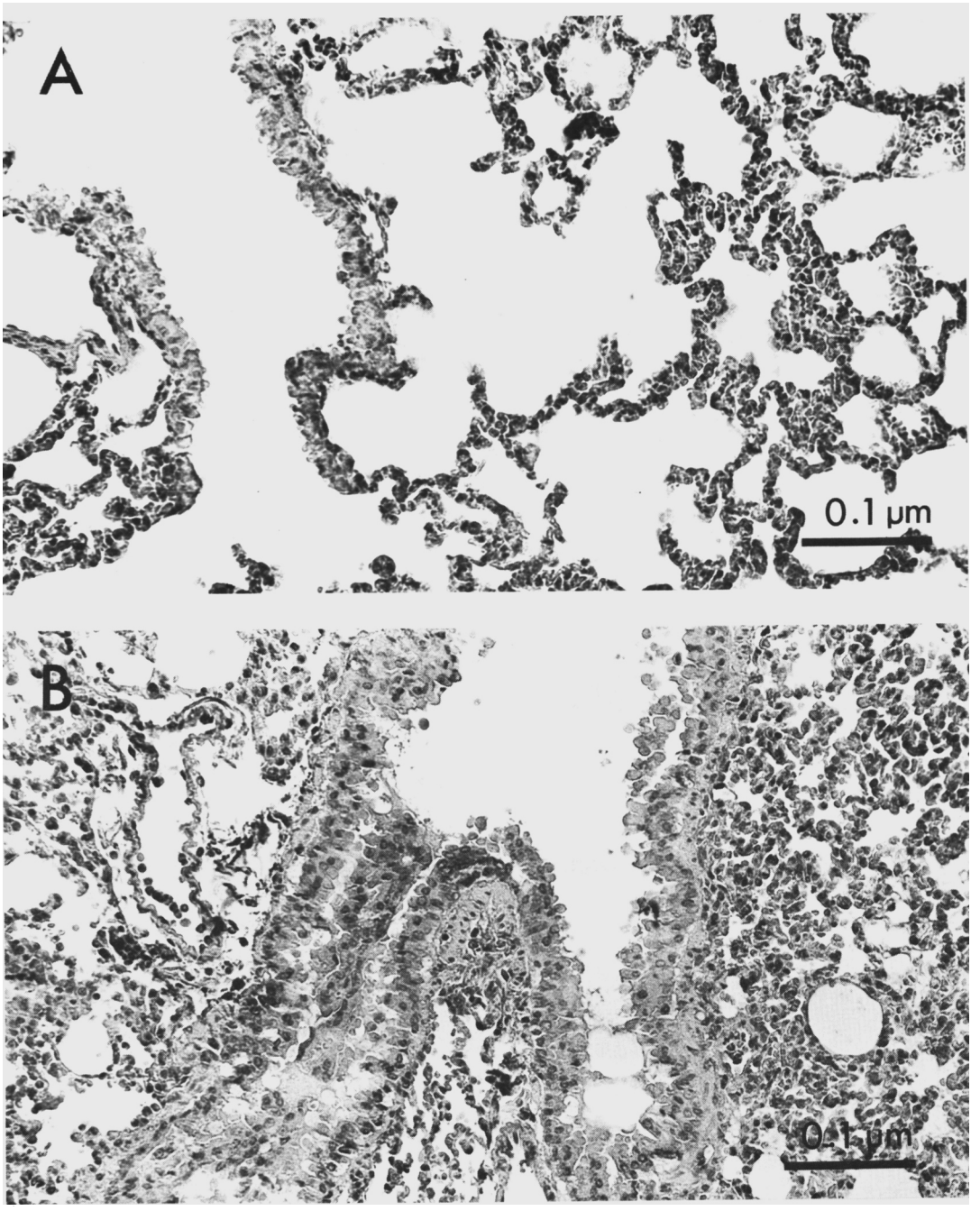

FIG. 1. Hematoxylin–eosin-stained section of adult mouse lung following intraperitoneal MAd1*pt*4 inoculation. (A) Lung section from an uninfected mouse. (B) Lung section obtained from an MAd1*pt*4-infected mouse after death. Note the extensive collapse of the alveolar structure, infiltration of mononuclear cells, and hyperplastic response of the bronchiole epithelium.

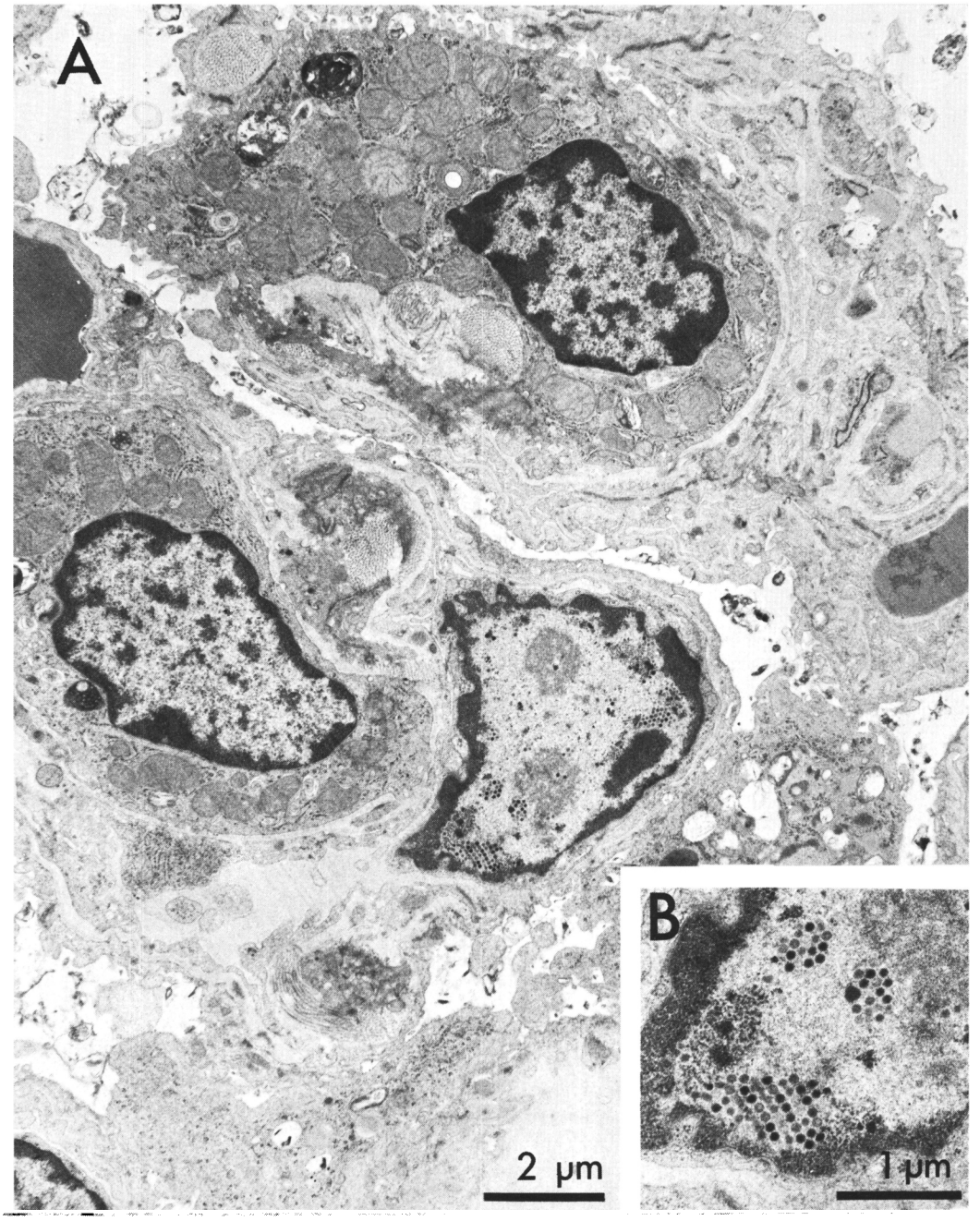

FIG. 2. Ultrathin sections of MAd1*pt*4-infected adult mouse lungs. (A) Low-magnification electron micrograph of an endothelial cell. Note the intranuclear particles. (B) High-magnification electron micrograph demonstrating adenovirus morphology.

TABLE II. SEROLOGIC RELATIONSHIP OF MAd1 AND MAd1*pt*4

Serum	Titer	
	MAd1 challenge	MAd1*pt*4 challenge
Control[a]	<50	<50
Anti-MAd1[b]	800	1600
Anti-MAd1*pt*4[c]	1600	1600

[a] Serum pool obtained from animal colony.
[b] Reference mouse adenovirus antiserum obtained from NCI.
[c] Serum obtained after MAd1*pt*4 infection.

as yet undefined gene responsible for pulmonary tropism. Isolation of additional plaque-type strains from MAd1, including additional defined border plaque isolations, will be necessary to address this question. On the basis of the notable pulmonary tropism of MAd1*pt*4, additional comparative studies of the plaque-type strains contained within MAd1 are warranted and might lead to the definition of adenovirus characteristics that are responsible for pulmonary tropism.

The well-characterized features of mouse immunogenetics coupled with the ability to perform experiments with relatively large mouse populations makes this animal a most suitable model for the study of adenovirus pathogenesis. Thus, MAd1*pt*4 presents a unique opportunity to develop a laboratory model for the comprehensive investigation of adenovirus pathogenesis involved in respiratory tract infection.

1. Binn, L. N., Eddy, G. A., Lazar, E. C., Helms, J., and Murnane, T., Proc. Soc. Exp. Biol. Med. **126**, 140 (1967).
2. Ditchfield, J., Macpherson, L. W., and Zbitnew, A., Canad. Vet. J. **3**, 238 (1962).
3. Campbell, R. S. F., Thompson, H., Cornwell, H. J. C., and Wright, N. G., Vet. Rec. **83**, 202 (1968).
4. Sharpe, H. B. A., and Jessett, D. M., J. Comp. Pathol. Ther. **77**, 45 (1967).
5. Shadduck, J. A., Koestner, A., and Kasza, L., Vet. Pathol. **4**, 537 (1967).
6. Darbyshire, J. H., Jennings, A. R., Dawson, P. S., Lamont, P. H., and Omar, A. R., Res. Vet. Sci. **7**, 81 (1966).
7. Eugster, A. K., Kalter, S. S., Kim, C. S., and Pinkerton, M. E. Arch. Ges. Virusforsch. **26**, 260 (1969).
8. Kim, C. S., Sueltenfuss, E. A., and Kalter, S. S., J. Infect. Dis. **117**, 292 (1967).
9. Ardans, A. A., Pritchett, R. F., and Zee, Y. C., Infect. Immunity **7**, 673 (1973).
10. McChesney, A. E., England, J. J., Adcock, J. L., Stackhouse, L. L., and Chow, T. L., Vet. Pathol. **7**, 547 (1970).
11. Norrby, E., Bartha, A., Boulanger, P., Dreizin, R. D., Ginsberg, H. S., Kalter, S. S., Kawamura, H., Rowe, W. P., Russell, W. C., Schlesinger, W. S., and Wigand, R., Intervirology **7**, 117 (1976).
12. Hartley, J. W., and Rowe, W. P., Virology **11**, 645 (1960).
13. Rowe, W. P., and Hartley, J. W., Ann. N.Y. Acad. Sci. **101**, 466 (1962).
14. Van der Veen, J., and Mes, A., Arch. Ges. Virusforsch. **45**, 386 (1974).
15. Wigand, R., Gelderblom, H., and Ozel, M., Arch. Virol. **54**, 131 (1977).
16. Margolis, G., Kilham, L., and Hoenig, E. M., Amer. J. Pathol. **75**, 363 (1974).
17. Hoenig, E. M., Margolis, G., and Kilham, L., Amer. J. Pathol. **75**, 375 (1974).
18. Ginder, D. R., J. Exp. Med. **120**, 1117 (1964).
19. Van der Veen, J., and Mes, A., Arch. Ges. Virusforsch. **42**, 235 (1973).
20. Lawrence, W. C., and Ginsberg, H. S., J. Virol. **1**, 851 (1967).
21. Reed, L. J., and Muench, H., Amer. J. Hyg. **27**, 493 (1938).
22. Winters, A. L., and Brown, H. K., Proc. Soc. Exp. Biol. Med. **164**, 280 (1980).
23. Cooper, P., Advan. Virus Res. **8**, 319 (1961).
24. Spencer, H., "Pathology of the Lung," Vol. 1. Pergamon Press, New York (1977).

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