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Duodenal Lesions Associated with Adenovirus Infection in Athymic "Nude" Mice (40862)

ALVIN L. WINTERS AND H. KEITH BROWN

Departments of Medical Microbiology and Anatomy, College of Medicine, University of South Florida, Tampa, Florida 33612

Abstract. Type 1 mouse adenovirus has been associated generally with nonlethal infections in adult mice. In contrast, athymic (nude) adult mice developed a lethal wasting disease following inoculation with type 1 mouse adenovirus (Strain FL). A characteristic duodenal hemorrhage occurred in the diseased mice immediately before death. Intranuclear inclusion bodies were observed in mucosal cells of the villi and crypts of the duodenum. Intranuclear adenovirus particles were observed in endothelial cells of the villi indicating that adenovirus replication was associated with the characteristic duodenal lesion. These observations indicate that type 1 mouse adenovirus should be considered as a candidate virus in the wasting disease syndrome of athymic (nude) mice.

Mouse adenovirus, a member of the Adenoviridae family (1), is species specific (2, 3). Two serotypes, MAd1 and MAd2, have been identified (4, 5). MAd2 was associated with a nonlethal and localized enteric infection, whereas MAd1 induced a systemic and frequently fatal disease in suckling mice (6–11). Inoculation of adult mice with MAd1 resulted in a systemic nonfatal disease characterized by development of neutralizing and complement fixing antibodies (12). The infectious virus has been recovered from thymus, spleen, leukocytes, lymph nodes, lungs, large intestine, kidneys, and urine. However, pathologic changes in intestinal tissue following MAd1 infection was not noted.

An apparent mouse adenovirus infection of congenitally athymic (nude) mice housed under conventional conditions has been reported by Cohen and de Groot (13). Large amphophilic inclusion bodies within swollen nuclei of enlarged mucosal cells located in the duodenum, jejunum, or ileum were noted in 30% of the mice that died spontaneously or were killed when moribund. Examination of these tissues by electron microscopy revealed clumps of adenovirus-like particles located within a homogeneous matrix of the nuclei. However, no evidence was presented showing that the adenovirus-like particles present in the intestinal tissue of the diseased mice were the specific causal agent for the morbidity and mortality of these mice. The present study demonstrated that MAd1 is an etiologic agent of lethal wasting disease in nude mice.

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, Rockville, Md.) maintained with Eagle's MEM medium supplemented with 5% fetal calf serum, streptomycin (0.1 mg/ml), and penicillin (100 units/ml). Virus titrations were performed on L-cell monolayer cultures in 60-mm dishes using a plaque assay (14) and infectivity was expressed as plaque forming units (pfu).

Animals and housing. Nude mice on an NIH Swiss and C3H/HeN background were supplied by Dr. Wendall Farrow (Life Sciences, St. Petersburg, Fla.) and Dr. Carl Hansen (National Cancer Institute, Bethesda, Md.), respectively. The parent mouse colonies were routinely negative for serologic evidence of the presence of mouse pathogens (specific pathogen free colonies) including mouse hepatitis virus and Sendai virus. Animals in this study were maintained in isolated quarters at 27° using aseptic procedures as described by Giovanella and Stehlin (15). Animals 4 to 6 weeks of age were inoculated aseptically with specified concentration of virus by ip injection of 0.5 ml of the virus in cell culture medium.

Light and electron microscopy. Fixation

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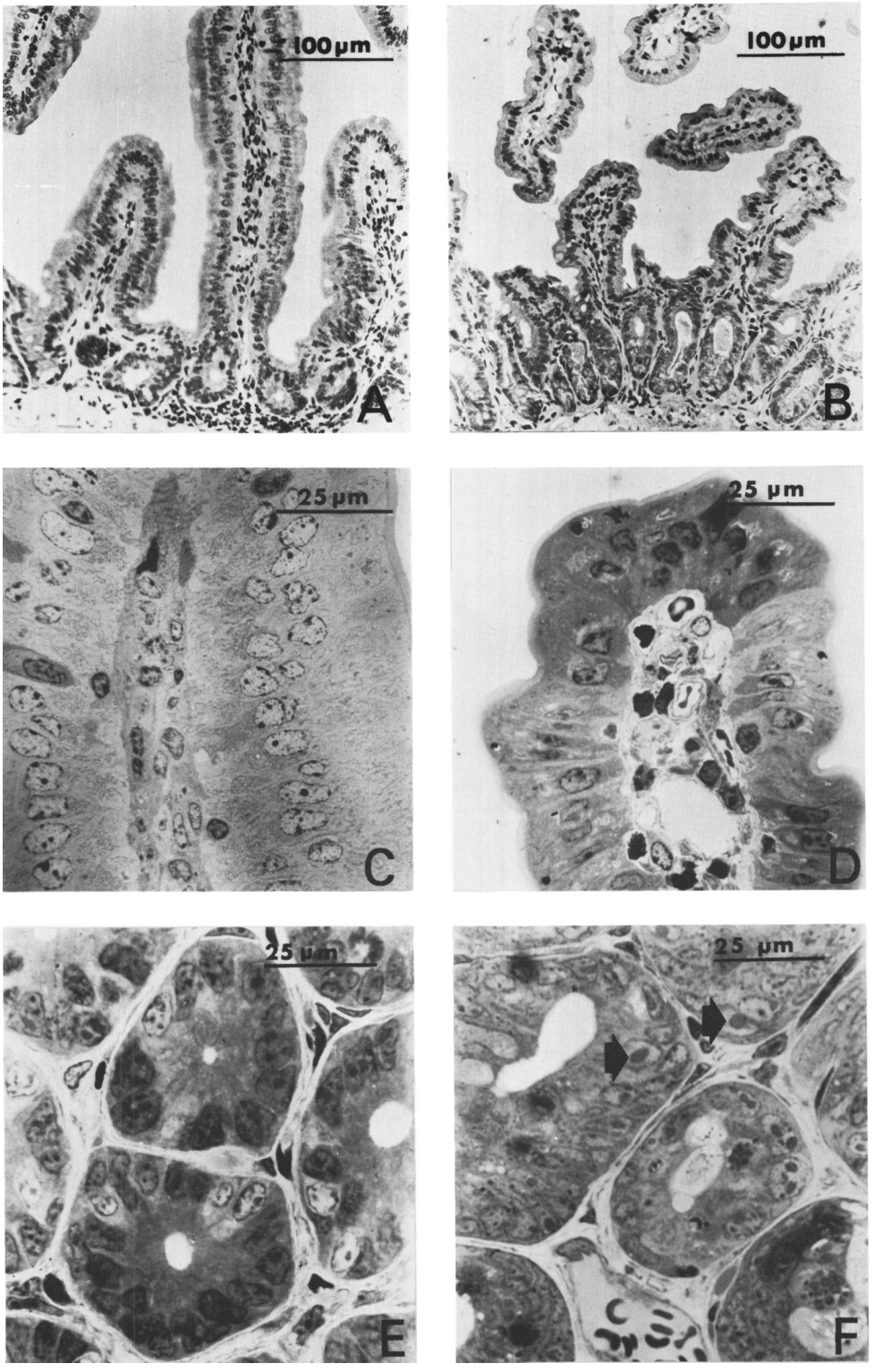
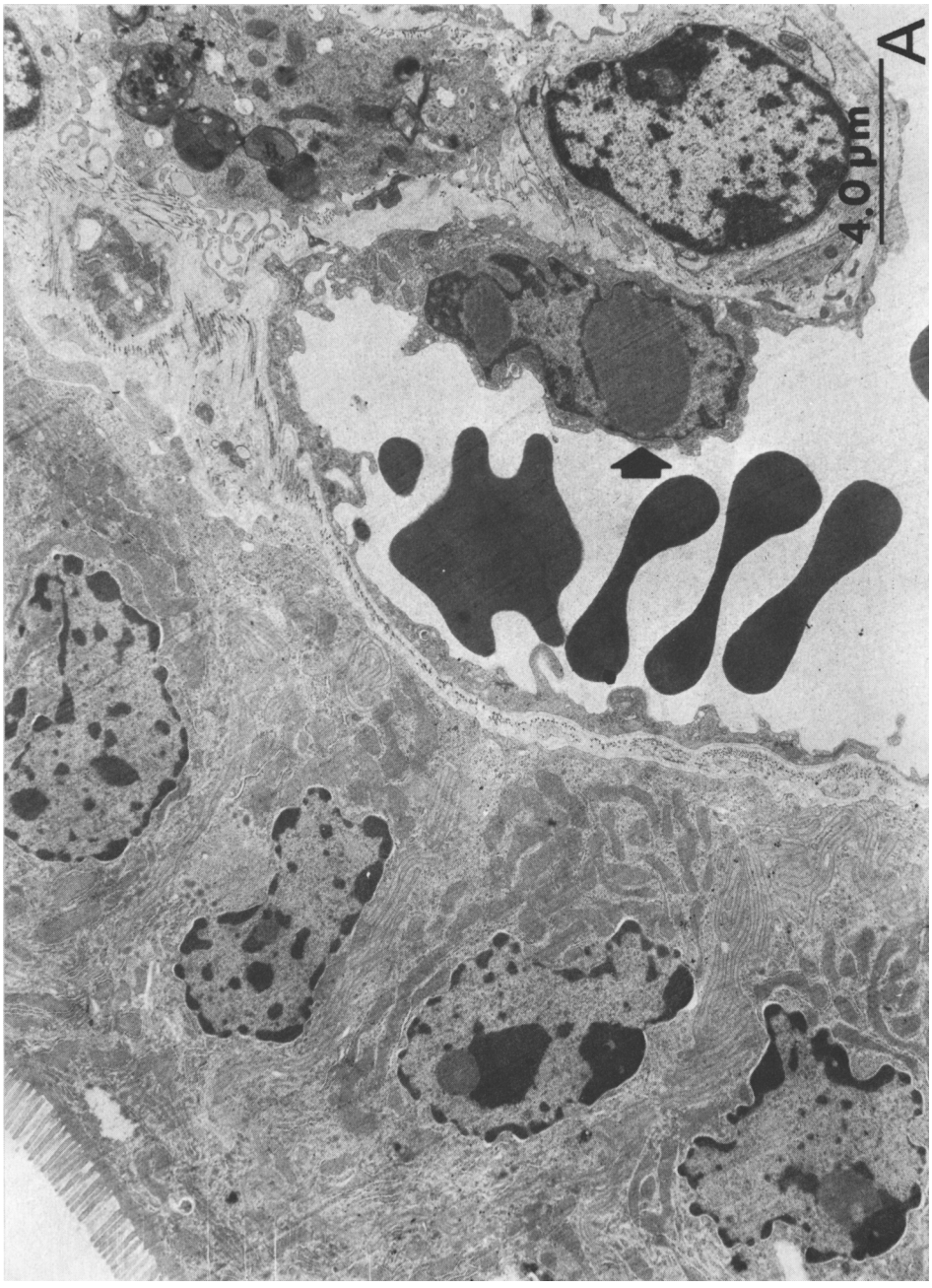


FIG. 1. Sections of *nu/nu* mouse duodenum. Hematoxylin–eosin stained sections of villi and crypts from an (A) uninfected mouse and (B) MAd1-infected mouse (note irregular mucosa and disrupted core of villi). Toluidine blue stained sections of villi from an (C) uninfected mouse and (D) MAd1-infected mouse (note the disrupted architecture of the infected villus). Toluidine blue stained sections of crypts from an (E) uninfected mouse and (F) MAdl-infected mouse (note the swollen nuclei and intranuclear inclusion bodies (arrows) in the cells from an infected animal).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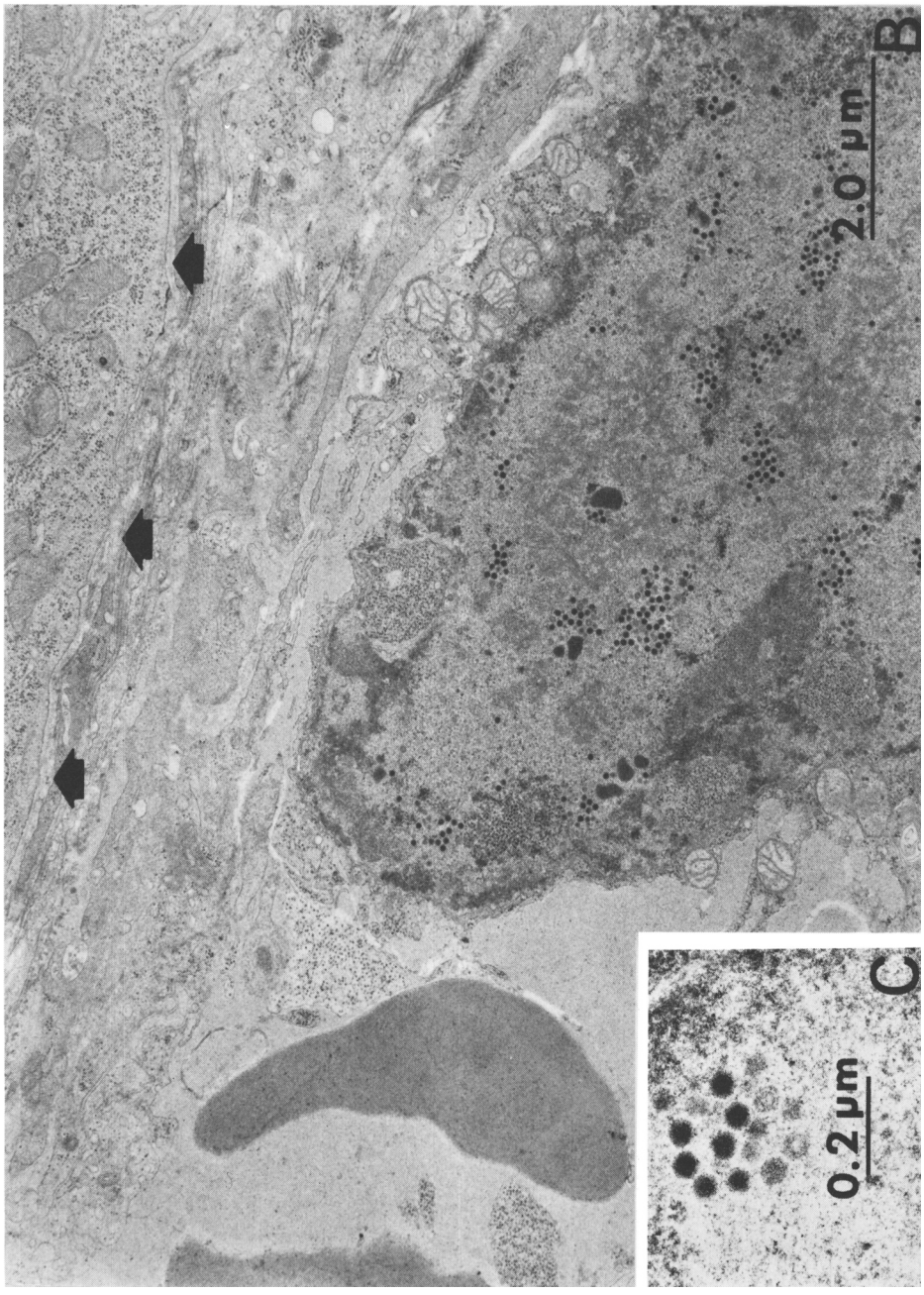

FIG. 2. Ultrathin sections of duodenum. (A) MAd1-infected villi (arrow denotes endothelial cell with an intranuclear inclusion body). (B) Endothelial cell with intranuclear adenovirus particles (arrows denote the basement membrane). (C) Higher magnification of the intranuclear adenovirus particles.

of the duodenum was performed by circulating fixative (2% of para-formaldehyde–3% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.3) directly through the lumen of the intestine and the left ventricle immediately after death (16). Two micrometer paraffin-embedded sections were processed with Mayer's hematoxylin–eosin stain (17). Specimens were also postfixed in 1.0% osmium tetroxide buffered with 0.1 *M* s-collidine, dehydrated, and embedded in Epon 812. One-micrometer sections were stained with toluidine blue. Ultrathin sections (60 to 70 nm) were stained with uranyl acetate and lead citrate.

Results. Virus dose–response in thymic and athymic mice. Congenitally athymic mice were notably susceptible to MAd1 infection. Inoculation of athymic homozygotic nude mice (*nu/nu*) and their thymus-bearing heterozygotic littermates (*nu/+*) with high doses of MAd1 (10^6 and 10^7 pfu) effected death in both *nu/nu* and *nu/+* mice within 5 days (Table I). Necropsy of both *nu/nu* and *nu/+* mice following high dose inoculation did not reveal pathologic changes at the macroscopic level. In contrast, *nu/nu* mice exhibited a notable susceptibility following low dose inoculation (10^5 pfu or less) as compared to *nu/+* mice. As little as 10^2 pfu resulted in "wasting disease" and death in *nu/nu* mice after a varied and sometimes prolonged period of time (Table I). Symptoms of "wasting disease" were a characteristic hunched back, drastic weight loss, and scaling skin. All the *nu/nu* mice inoculated with low virus doses had hemorrhaged into the lumen of the duodenum shortly before death, since the blood had not moved into the cecum and fecal pellet formation remained normal. Extensive examination of tissues other than the duodenum did not reveal recognizable lesions or alterations at the macroscopic level. In contrast, the *nu/+* mice inoculated with low virus doses (10^2 to 10^5 pfu) did not develop wasting disease or detectable pathologic changes.

Involvement of MAd1 in duodenal lesions. Infected and uninfected animals were necropsied immediately after death or sacrifice. The architecture of both the villi and crypts was abnormal in the infected animals (Figs. 1A, B). The lumen-facing surface was involuted, nuclear location was aberrant, and the core region of the villi appeared disrupted (Figs. 1C, D). Intranuclear inclusion bodies (eosinophilic) were observed in the crypt cells (Figs. 1E, F) and in the mucosal cells of the intestinal villi. Inclusion bodies were observed also in endothelial cells of lamina propria of villi (Fig. 2A). Higher magnification revealed adenovirus particles in a light staining amorphorus nuclear

TABLE I. MAd1 Dose–Response in Athymic "Nude" Mice and Heterozygotic Littermates

Genotype	Inoculum (pfu)	Mortality (deaths/total)	Time of death (days)	Macroscopic lesions[a] (No. observed)
NIH Swiss *nu/nu*	10^7	2/2	2,5	0
	10^6	2/2	2,3	0
	10^5	2/2	28,15	2
	10^4	2/2	10,11	2
	10^3	2/2	72,134	2
	10^2	2/2	69,45	2
C3H/HeN *nu/nu*	10^5	5/5	38,42,54,54,60	5
NIH Swiss *nu/+*	10^7	2/2	2,3	0
	10^6	2/2	2,3	0
	10^5	0/2	–,–	0
	10^4	0/2	–,–	0
	10^3	0/2	–,–	0
	10^2	0/2	–,–	0
C3H/HeN *nu/+*	10^5	0/7	–,–,–,–,–,–,–	0

[a] Hemorrhage into the lumen of the duodenum, blood extended to near the cecum, and feces formation appeared normal in the large intestine.

matrix of an occasional endothelial cell (Figs. 2B, C).

Discussion. The mice used in this study were obtained from two independent sources, placed in rooms that had not previously housed mice or other animals, and maintained using aseptic procedures. The MAd1 associated wasting disease, mortality, and characteristic pathologic changes described in this report has never been observed in uninfected *nu/nu* mice over a 4-year period. Thus, the inoculated MAd1 was directly associated with the intestinal disease that developed in nude mice. Although tissues other than the duodenum may have been involved in the disease process, the close chronological association between duodenal hemmorhage and death implies that the lesions in the duodenum were intimately involved in the lethal disease process. Demonstration of intranuclear inclusion bodies characteristic of adenovirus cytopathology and particles with adenovirus morphology in the nuclei of cells of intestinal tissue also strongly supported the view that MAd1 replication was involved in the duodenal lesions. Although intranuclear inclusion bodies were relatively numerous in the mucosal cells of the villi and crypts, intranuclear adenovirus particles were observed only in a limited number of endothelial cells. These data suggest that limited virus replication in the duodenal endothelial cells effects cytopathologic changes in the mucosal cells.

Thymus function appears to play an important role in resistance to adenovirus infection. A possible mechanism of resistance may be the thymus-derived lymphocyte. Lymphocyte cytotoxicity directed toward MAd1 infected target cells has been demonstrated (18). The ability of *nu/nu* mice to tolerate MAd1 infections for extended periods of time (up to 134 days) suggested that either low levels of thymus function were present in *nu/nu* mice or that other factors, such as macrophages, antibodies, or interferon, may have limited the viral-induced pathologic changes. The factors which permit *nu/nu* mice to survive MAd1 infection for extended periods of time warrants additional studies.

In searching for the origin of MAd1 contamination in *nu/nu* mouse colonies, one should consider possible sources from several months in the past. Conversely, observations of animal deaths in a colony due to MAd1 probably indicates widespread infection, since the virus probably entered the colony several months before deaths were observed. Observation of duodenal hemorrhage upon necropsy of *nu/nu* mice might provide a convenient indicator of MAd1 infection, however additional studies will have to be performed to establish the diagnostic value of this observation. In addition to mouse hepatitis virus (19, 20) and Sendai virus (21–23), MAd1 should be considered also as a candidate virus in the wasting disease syndrome of *nu/nu* mice.

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1. 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., Invervirology 7, 117 (1976).
2. Hartley, J. W., and Rowe, W. P., Virology **11**, 645 (1960).
3. Rowe, W. P., and Hartley, J. W., Ann. N.Y. Acad. Sci. **101**, 466 (1962).
4. Van der Veen, J., and Mes, A., Arch. Gesamte Virusforch. **45**, 386 (1974).
5. Wigand, R., Gelderblom, H., and Ozell, M., Arch. Virol. **54**, 131 (1977).
6. Sugiyama, T., Hashimoto, K., and Sasaki, S., Japan. J. Microbiol. **11**, 33 (1967).
7. Heck, F. C., Jr., Sheldon, W. G., and Gleiser, C. A., Amer. J. Vet. Res. **33**, 841 (1972).
8. Blailock, Z. R., Rabin, E. R., and Melnick, J. L., Science **157**, 69 (1967).
9. Blailock, Z. R., Rabin, E. R., and Melnick, J. L., Exp. Molec. Pathol. **9**, 84 (1968).
10. Margolis, G., Kilham, L., and Hoenig, E. M., Amer. J. Pathol. **75**, 363 (1974).
11. Hoenig, E. M., Margolis, G., and Kilham, L., Amer. J. Pathol. **75**, 375 (1974).
12. Van der Veen, J., and Mes, A., Arch. Gesamte Virusforch. **42**, 235 (1973).
13. Cohen, B. J., and de Groot, F. G., Lab. Animal. Sci. **26**, 955 (1976).
14. Lawrence, W. C., and Ginsberg, H. S., J. Virol. **1**, 851 (1967).

15. Giovanella, B. C., and Stehlin, J. S., J. Nat. Cancer Inst. **51**, 615 (1973).
16. Brown, H. K., and Nolan, M. F., J. Neurocytol. **8**, 167 (1979).
17. "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology" (L. G. Luna, ed.), The Blakison Division, 3rd ed. McGraw–Hill, New York (1968).
18. Inada, T., and Uetake, H., Infect. Immun. **20**, 1 (1978).
19. Hirano, N., Tamura, T., Taguchi, F., Ueda, K., and Fujiwara, K., Japan. J. Exp. Med. **45**, 429 (1975).
20. Sebesteny, A., and Hill, A. C., Lab. Anim. **8**, 317 (1974).
21. Ward, J. M., Houchens, D. P., Collins, M. J., and Reagan, R. L., Vet. Pathol. **13**, 36 (1976).
22. Ueda, K., Tamura, T., and Fujiwara, K., Proc. Int. Workshop Nude Mice, 2nd, 61 (1976).
23. Iwaskai, Y., *in* "The Nude Mouse in Experimental and Clinical Research" (J. Fogh and B. C. Giovanella, eds.), p. 457. Academic Press, New York (1978).

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