

Measles Virus Replication in Lungs of Hispid Cotton Rats after Intranasal Inoculation (43483)

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Abstract. Hispid cotton rats were inoculated intranasally with either measles virus (MV) Edmonston, a multipassaged, tissue culture-adapted strain of MV, or with one of three clinical MV isolates that had limited passages (three to five times) in tissue culture cells. MV Edmonston was recovered from the lungs of every ($n = 37$) hispid cotton rat inoculated with this virus for at least 7 days after virus inoculation. Peak pulmonary titers occurred on Day +4 (3.3–4.4 log₁₀/g lung). Scattered areas of inflammation were observed interstitially in lung sections from infected animals stained with hematoxylin and eosin, and a similar pattern of diffuse fluorescence was seen in cryostat sections stained with an indirect fluorescent antibody procedure specific for virus antigens. Fluorescent antibody and virus isolation studies on lung lavage cells both suggested that lung leukocytes were a primary target of the virus. In contrast to these findings, virus was isolated only sporadically from hispid cotton rats inoculated with any of the clinical measles virus isolates. Despite the restricted growth of MV in these animals, cotton rats may be useful for studying certain aspects of measles virus pathogenesis and for screening potential antiviral compounds *in vivo*. [P.S.E.B.M. 1992, Vol 201]

Despite the availability of efficacious vaccines, measles continues to have a staggering impact worldwide. Over 1 million people, predominantly children, die each year from measles virus-induced pneumonia or related complications (1). Moreover, measles virus (MV) can disseminate from the lung and cause an array of debilitating disorders of the central nervous system (CNS), liver, pancreas, and immune system (2–9).

The continuing impact of measles has resulted in a renewed interest in the study of the epidemiology, pathogenesis, and control of measles virus (1, 10, 11). It has also led to a reevaluation of available vaccines and their distribution (10) and to a new interest in identifying and developing compounds with selective anti-MV activity (12, 13).

In vivo investigations of measles have been limited primarily due to the lack of a practical animal para-

digm. Monkey models have long been favored for *in vivo* measles studies because these primates often develop a disease similar to that seen in humans after experimental inoculation (14–16). However, high cost, limited availability, and widespread restrictions on the use of primates severely limit the use of monkeys for all but the most important studies. Rodents are much less costly, are readily available, and have fewer restrictions on their use; and indeed, MV-rodent models (i.e., newborn rats, mice, and hamsters) have been developed (17–19). However, these models have restricted use because of the physiological immaturity and small size of newborn rodents, and because virus inoculation and replication is limited to the CNS.

Cotton rats have been used to study the pathogenesis of respiratory syncytial virus (RSV) and parainfluenza virus type 3 ([PIV3] 20–22). Although related to these viruses, no reports describing the ability of MV to grow in cotton rats are known to us. However, because of the relatedness of MV to PIV3 and RSV, it is plausible that MV could grow in these animals. The following report summarizes findings indicating restricted, but significant, growth of a high-passage, tissue culture-adapted MV strain (Edmonston) in lungs of cotton rats after intranasal inoculation. Possible utilizations of a cotton rat-MV model are discussed.

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Materials and Methods

Animals. The *Sigmodon hispidus* cotton rats used in these studies were bred from several pairs acquired with the help of Dr. Gregory Prince from the Small Animal Section, Veterinary Research Branch, Division Research Services, National Institutes of Health, Bethesda, MD. All animals were 4 to 8 weeks old (40–100 g) at the start of the experiments and of either sex. All animals were housed in cages with barrier filters and given food and water *ad libitum*.

Tissue Culture. Vero (African green monkey kidney; American Type Culture Collection [ATCC] CCL81) and HEP-2 (ATCC CCL 23) tissue culture cells were obtained initially from the ATCC. Both cell lines were propagated in monolayer culture in Earle's minimum essential medium supplemented with fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 μ /ml), sodium bicarbonate (0.2%), and L-glutamine (2 mM/ml). Each cell line was serially passaged whenever they became confluent.

Viruses. The Edmonston strain of measles virus was acquired from the ATCC (Cat. No. VR24). This virus was originally isolated from the blood of a patient in the acute phase of typical measles by Dr. John Enders (23) and then passaged >40 times in human kidney and amniotic tissue culture cells before being transferred to the ATCC. The three clinical isolates of MV used in these studies, 11233, 11498, and AC705, were obtained from E.C. Herrmann, Ph.D. (Mobilab, Peoria, IL) and Gail Demmler, M.D. (Department of Pediatrics, Baylor College of Medicine). All three clinical virus isolates were obtained from throat cultures of patients in the acute phase of typical measles. Each was initially isolated in primary monkey kidney cells and then passaged two to four times in Vero cells prior to inoculation of cotton rats.

Working stocks of MV were prepared by infecting monolayers of Vero cells and incubating the cultures in a CO₂ (5%) incubator maintained at 35°C. When cytopathic effects (CPE) in these cultures were 80–100% evident (usually 5 to 7 days), the medium and cells in each culture were collected, placed in a Branson sonicating water bath (model B-220) and exposed to six 15-sec bursts of sonication (50/60 Hz). Each preparation was then centrifuged (450g) to remove particulate matter. The resulting supernatants were filtered through a 0.45- μ m filter (Acrodisk, Cat. No. 4184; Gelman, Ann Arbor, MI), portioned, labeled, and stored at -70°C.

Inactivated MV was prepared by placing suspensions of virus 35 mm below an activated 30 W ultraviolet (UV) light source (GTE Sylvania) for 7 min. The exposed suspensions were collected, filtered through a 0.45- μ m filter, and tested for virus viability, as described below.

The RSV A2 and PIV3 used in these studies were obtained from the ATCC (Cat. Nos. VR1302 and VR93, respectively). Working stocks of these viruses were prepared in a manner similar to that described for the preparation of MV stocks, except that they were grown in HEP-2 tissue culture cells.

Confirmation of Virus Identity. Replication of each of the MV strains was totally inhibited in microneutralization assays by rabbit antisera to MV (Cat. No. 8541; Lee Biomolecular Research, Inc., San Diego, CA) at concentrations \geq 1:80, but not by antisera specific to RSV (Cat. No. 06401; Lee Biomolecular) or PIV3 (Cat. No. 30-870N; Whittaker Bioproducts) at concentrations >1:4. The RSV and PIV3 were similarly neutralized by antiserum specific to them at concentrations \geq 1:80, but not by either heterologous antisera at concentrations of >4.

Collection of Samples. Blood was collected from the orbital sinus plexus. After clotting and centrifugation, sera were collected and heat inactivated at 56°C for 30 min. The animals were then asphyxiated using CO₂. Lungs from these animals were removed, weighed, and transpleurally lavaged, as described in detail elsewhere (24). The resulting lung cell suspensions were centrifuged at 480g and separated into fluid and cellular fractions. The latter fractions were resuspended to their original volumes in 2% FCS-RPMI 1640 medium. All samples were kept on ice until assayed, usually within 1 hr of collection.

Lungs designated for histological processing and evaluation were collected as described above and placed in Omnifix fixative (Zymed Laboratories, Inc., San Francisco, CA) for at least 24 hr. These lungs were then processed and evaluated as described below.

Virus Quantification. Routinely, lung lavage suspensions were assessed for the presence and levels of infectious virus in 96-well microassays using Vero (MV) or HEP-2 (RSV and PIV3) tissue culture cells and CPE as an end point. In these assays, each test sample was serially diluted in 2% FCS-RPMI 1640 medium using 0.5 log₁₀ dilutions. Approximately 2×10^4 Vero cells in 100 μ l of RPMI medium were then added to each well. All plates were incubated at 35°C and observed daily for syncytia formation. Final observations and recording of CPE were usually made on Day 7 of each assay. Geometric mean pulmonary virus titers (GMPT) were obtained by determining the maximal dilution of each replicate sample that contained virus and calculating the geometric mean (log₁₀/g lung) for each test group.

The amount of virus in inocula was expressed as the quantity of virus that could infect 50% of inoculated tissue culture monolayers (TCID₅₀) or cotton rats (CRID₅₀). TCID₅₀ values were determined by preparing serial 0.5 log₁₀ dilutions of virus in medium and adding in quadruplicate 0.05 ml of each dilution to monolayers

of Vero or HEp-2 cells. At the end of a 7-day incubation period, all wells were observed and the maximal dilution of virus that could infect one half the inoculated replicate monolayers was determined using the procedure of Karber (25). CRID₅₀ values were determined by preparing serial log₁₀ dilutions of virus in medium and inoculating each of four animals intranasally with 0.1 ml of each dilution. On Day +4, all animals were sacrificed and assessed for pulmonary virus. The maximal dilution of virus that could cause pulmonary infection in one-half the cotton rats inoculated was determined using the Karber method (25). Because clinical MV isolates could only be recovered from the lungs of experimentally inoculated cotton rats intermittently, the CRID₅₀ was only determined for RSV, PIV3, and Edmonston MV.

Serum Antibody Assay. Virus-specific serum-neutralizing antibody levels were determined in neutralization tests performed in 96-well tissue culture plates. Briefly, test antisera were diluted in 2% FCS-minimum essential medium using serial 2-fold dilutions. Equal volumes (50 μ l) of MV containing approximately 100 TCID₅₀ of virus were then added to each well. After a 90-min incubation at room temperature, 100 μ l of Vero cells (approximately 2×10^4 cells) were added to each well, including control wells containing neither antisera nor virus (tissue control) and control wells containing virus but no antisera (virus control). The plates were placed in a 35°C (5%) CO₂ incubator and observed daily. When monolayers in the virus control wells exhibited >70% CPE, all wells were observed. Titers were expressed as log₂ of the reciprocal of the last dilution of antiserum that inhibited virus-induced CPE 100%.

Histological Methods and Evaluations. Lung tissues collected for histopathological evaluations were fixed in Omnifix for a minimum of 24 hr, embedded in low-melting point paraffin, sectioned at 5 μ m thickness, and stained with hematoxylin and eosin. The stained sections were observed in a blinded fashion for histopathology using a light microscope.

Frozen thin sections were prepared by immersing the lungs in O.C.T. compound (Ames Co., Eckhart, IN) and then quickly freezing by using a dry ice in ethanol mixture. Cryostat sections were cut at 10 μ m and kept at -20°C until stained and observed.

In a number of experiments, suspensions of lung lavage cells were placed in a cytocentrifuge (Shandon-Elliot cytopsin) and pelleted onto glass slides. The pelleted cells were then stained either with a combination of eosin Y and methylene blue (Leuko-stat stain; Fisher Diagnostics, Orangeburg, NY), or with indirect fluorescent antibody-staining reagents.

Immunofluorescence Studies. Frozen thin sections or cells pelleted onto slides in a cytocentrifuge were fixed in cold acetone and stained using an indirect fluorescent antibody technique designed to detect spe-

cific viral antigens. Briefly, the preparations were washed in phosphate-buffered saline and overlaid with rabbit antiserum to MV (1:40 in phosphate-buffered saline) for 30 min. The slides were then washed and overlaid for 30 min with fluorescein isothiocyanate-conjugated goat antiserum to rabbit IgG (Cappel Cat. No. 1212-0081). After a final wash in phosphate-buffered saline, the stained preparations were mounted and observed using a Leitz Wetzlar Ortholux II UV microscope equipped with a HBO-200W mercury burner, a BG12 excitation filter, and a 470 barrier filter.

Statistics. Determination of means, standard deviations, and *P*-values using Student's *t* test and other data analysis was performed using True Epistat, a statistical program designed by T. L. Gustafson of Epistat Services, Richardson, TX, for IBM-compatible computers.

Results

Pulmonary Virus Isolation. In these experiments, virus was isolated from the lungs of every hispid cotton rat inoculated with MV Edmonston for at least 7 days after virus inoculation (Table I). Maximal geometric mean pulmonary titers occurred on Day +4 (GMPT = 3.3 log₁₀/g lung). However, actual virus isolation rates were dependent upon the lung fraction tested for virus. Thus, virus was recovered from 74% of the lung homogenates tested on Day +4 (GMPT = 3.0 log₁₀/g lung), from 100% of the cellular fractions of lung lavage cell suspensions (GMPT = 3.3 log₁₀/g lung), and from only 26% of the fluid fractions of lung lavage cell suspensions (GMPT = 1.1 log₁₀/g lung). Using Student's *t* test to compare either percentage of virus isolation or GMPT, no statistical differences in the virus isolation rates, or GMPT, were observed between lung homogenates or the cellular fractions of lung lavage

Table I. Comparison of Virus Recovery from Different Lung Specimens after Intranasal Inoculation of Hispid Cotton Rats with Edmonston MV^a

Specimen tested	Samples virus positive/sample tested (n)	Virus positive (%)	GMPT/g lung ^b (log ₁₀)
Lavage fluids (-cells)	10/38	26	1.1 ± 0.7
Lung lavage cells	38/38	100	3.3 ± 0.4
Lung homogenate	23/31	74	3.0 ± 1.1

^a Cotton rats were lightly anesthetized with ether and inoculated intranasally with approximately 100 CRID₅₀ doses of virus in 100 μ l. On Day +4 after virus inoculation, all animals were sacrificed and the lungs from these cotton rats were either homogenized or lavaged. The homogenates and cellular and fluid fractions of the lavage fluids (obtained by centrifugation at 480g) were tested in Vero cells for measles virus.

^b Geometric mean pulmonary titer (GMPT) ± SD; lungs with undetectable virus were assigned a value of 0.8 log₁₀ for statistical evaluation; underlined values indicate GMPT significantly different from GMPT obtained for lung lavage cells.

suspensions ($P > 0.05$); however, the virus isolation rates and GMPT for homogenates and the cellular lung lavage fractions were both significantly greater than that obtained using the fluid fractions of lung lavage suspensions ($P < 0.05$). Although the percentages of virus isolation and GMPT obtained using lung homogenates were not significantly different from those using the cellular portions of the lung lavage fluids, lung lavage cells were used in all subsequent experiments because it was considerably easier to read wells containing lung lavage suspensions for virus-induced CPE than those containing lung homogenates.

In contrast to the high rate of virus recovery from the lungs of hispid cotton rats inoculated intranasally with Edmonston MV, virus was isolated from only two of 10 hispid animals inoculated similarly with the clinical MV isolate, AC705, and from none of the animals inoculated with clinical isolates 11233 and 11498 (Table II). The GMPT in the two cotton rats from which AC705 virus was isolated was $3.6 \pm 0.4 \log_{10}/g$ lung, not significantly different from the GMPT observed in animals inoculated with Edmonston MV ($4.4 \pm 0.3 \log_{10}/g$ lung; $P > 0.05$ using Student's t test).

Confirmation of Virus Identity. The identity of the viruses recovered from the lungs of experimentally inoculated cotton rats was verified periodically in serum neutralization assays. In these assays, all of the recovered virus isolates were completely inhibited by rabbit antiserum specific for measles at dilutions $\geq 1:80$, but not by antiserum specific for PIV3 or RSV at dilutions $> 1:4$. The distinctive "neuronal" type syncytia induced by the virus isolates in Vero cells (Fig. 1) provided additional evidence that the recovered viruses were MV; this distinctive syncytia has only been observed in this laboratory with Edmonston MV and a limited number of clinical isolates of MV.

Kinetics of Virus Replication. Figure 2 compares the kinetics of replication of Edmonston MV and RSV in lungs of cotton rats after intranasal inoculation

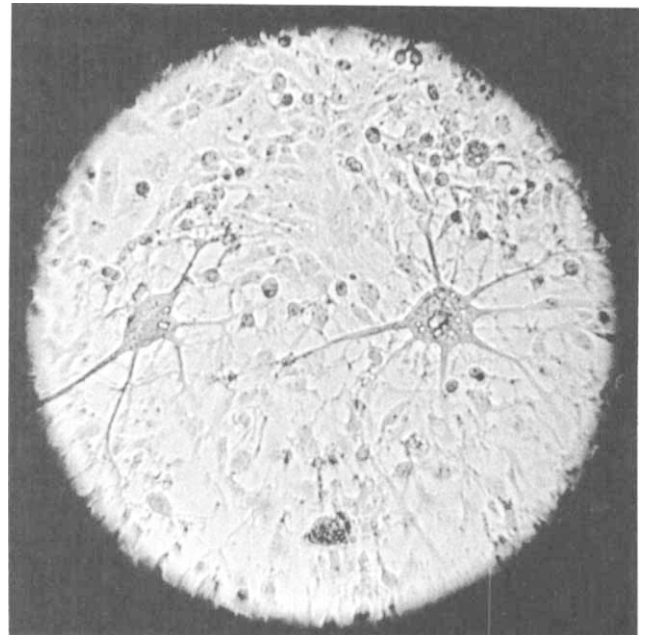


Figure 1. Monolayer of Vero cells 3 days after inoculation with lung lavage suspension cells obtained from a cotton rat 4 days after the animal was inoculated intranasally with measles virus Edmonston. Note formation of distinctive "neuronal" type syncytia.

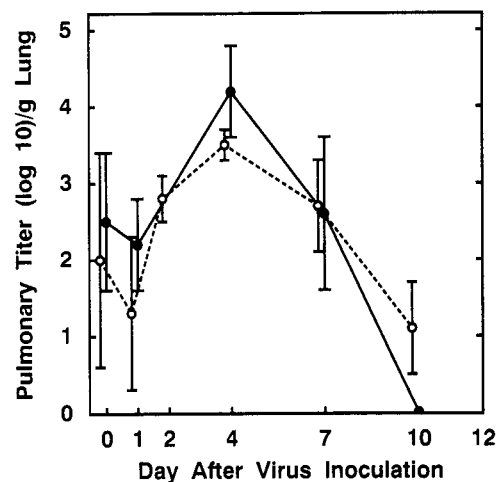


Figure 2. Comparison of the kinetics of measles (dashed line) and respiratory syncytial (solid line) virus replication in lungs of cotton rats inoculated intranasally with approximately 100 CRID₅₀ doses of the appropriate virus.

Table II. Comparison of Virus Recovery from Lungs of Hispid Cotton Rats Intranasally Inoculated with Different MV Strains and Isolates^a

Measles virus inoculate	Animals positive/animals inoculated (n)	All animals inoculated ^b	Only positive animals ^b
Edmonston	6/6	4.4 ± 0.3	4.4 ± 0.3
Isolate 11498	0/6	0	0
Isolate AC705	2/10	0.8 ± 1.2	3.6 ± 0.4
Isolate 11233	0/6	0	0

^a Cotton rats were lightly anesthetized with ether and inoculated intranasally with approximately 10^3 TCID₅₀ doses of the appropriate virus in 100 μ l. On Day +4 after virus inoculation, all animals were sacrificed and the lungs were tested in Vero cells for measles virus.

^b Geometric mean pulmonary titer (GMPT) \pm SD.

of these animals with approximately 100 CRID₅₀ of the appropriate virus. As indicated, both paramyxoviruses replicated in the lungs of inoculated animals, reached maximum pulmonary levels 4 days after virus inoculation, and were absent or minimally present 7–12 days after inoculation. The maximum GMPT seen for the Edmonston MV in this experiment was $3.5 \log_{10}/g$ lung compared with peak pulmonary titers of $4.6 \log_{10}/g$ lung for RSV. In eight different experiments, the GMPT on Day +4 from cotton rats experimentally inoculated

intranasally with Edmonston MV ranged from 3.3 to 4.6 log₁₀/g lung.

Histological Findings. Scattered foci of mild inflammation, usually consisting of small numbers of mixed populations of leukocytes (i.e., lymphocytes, macrophages, and polymorphonuclear leukocytes), were observed in hematoxylin-and-eosin-stained sections of lung prepared from cotton rats sacrificed on Days 4 and 7 after intranasal inoculation of these animals with Edmonston MV (Fig. 3, B, C, and D). Such foci were rarely seen in sections of lung from uninoculated, sham-inoculated control animals, or cotton rats inoculated with any of the clinical MV isolates (Fig. 3A). The inflammatory foci were predominantly interstitial and seldom involved bronchi or bronchioles. Few inflammatory foci were evident on Day +10.

As described in Materials and Methods, lung cells obtained by transpleural lung lavage were often pelleted onto glass slides using a cytocentrifuge and stained with LeukoStat stain for differential counting. An overwhelming number (>95%) of these cells, whether they were obtained from sham-inoculated or virus-inoculated animals, were mononuclear and polymorphonuclear leukocytes (Fig. 4, A and B). However, in contrast to the lung lavage cells obtained from sham-inoculated control animals (Fig. 4A), the majority of leukocytes from MV-inoculated cotton rats (Fig. 4B) exhibited both increased cytoplasmic to nuclear ratios and a marked cytoplasmic vacuolization, signs of cell activation.

Fluorescent Antibody Studies. Scattered foci of fluorescence were observed on Days +4 and +7 in frozen sections of lung obtained from cotton rats inoculated with MV Edmonston and stained with an indi-

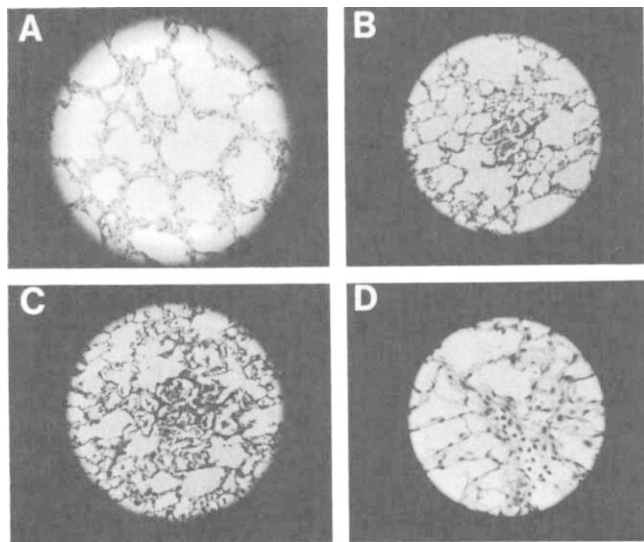


Figure 3. Section of lung from (A) a sham-inoculated cotton rat and from (B–D) three cotton rats 4 days after intranasal inoculation with measles virus Edmonston. Note small foci of interstitial inflammation in B through D.

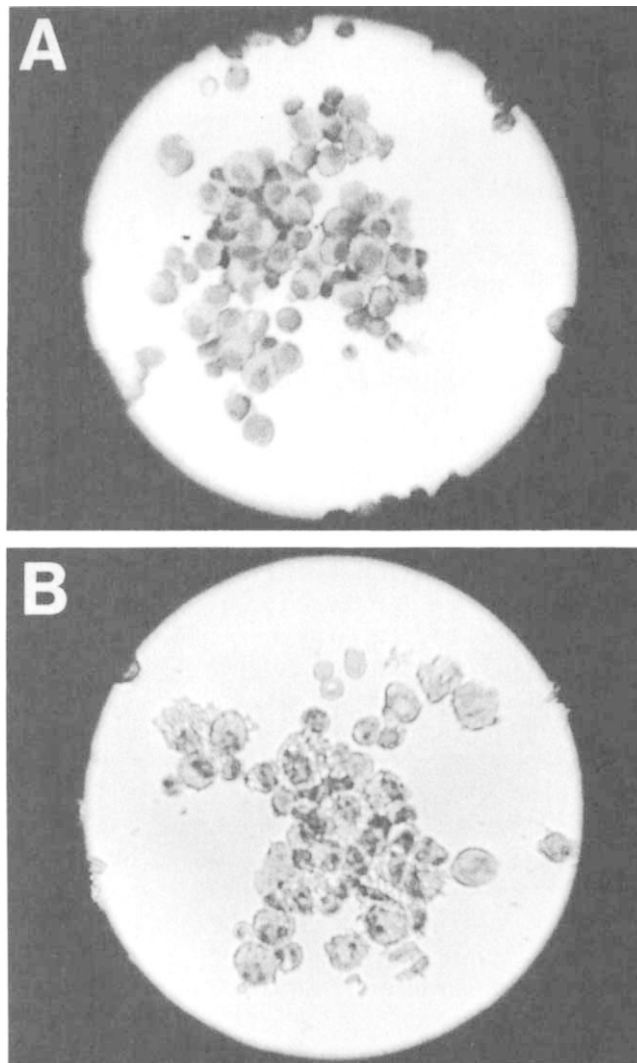


Figure 4. Lung lavage cells stained with eosin Y and methylene blue (Fisher LeukoStat stain) after being pelleted onto glass slides using a cytocentrifuge. (A) Lung lavage cells from a sham-inoculated cotton rat 4 days after inoculation. Most cells are leukocytes with relatively unvacuolated cytoplasm. (B) Lung lavage cells from a cotton rat 4 days after inoculation with the Edmonston strain of measles virus. Most cells are leukocytes with vacuolated cytoplasm and an apparently increased cytoplasmic to nuclear ratio.

rect fluorescent antibody procedure for the presence of MV-specific antigens. These fluorescing foci were not evident in sections of lung obtained from sham-inoculated control animals and were less evident in sections of lung from animals harvested on Days +2 or +10 after virus inoculation. The areas of fluorescence were generally small, scattered interstitially throughout the lungs, and did not photograph well. However, the pattern of fluorescence did coincide well with the pattern of scattered foci of inflammation seen in sections of lung stained with hematoxylin and eosin. Because no similar fluorescence was observed in preparations stained in the primary staining step with antiserum specific to PIV3 or RSV, nor in frozen sections of lung

obtained from uninoculated animals and stained with serum specific for MV, the fluorescence appeared to be MV-specific.

Lung lavage cells obtained from both control and virus-infected animals and pelleted onto glass slides by cytocentrifugation were also stained using the indirect fluorescent antibody procedure. When these stained slides were viewed through a fluorescence microscope, numerous bright apple green (virus-positive) cells were observed on slides containing cells from the MV-infected, but not sham-inoculated, cotton rats (see Fig. 5, A-C). The fluorescence appeared limited to mononuclear cells, was cytoplasmic (most evident in Fig. 5B), and was not evident if heterologous sera (i.e., sera to RSV or PIV3) or MV-specific antisera produced in a different species (i.e., cotton rats) were utilized before the addition of fluorescein isothiocyanate-conjugated anti-rabbit sera. Maximum numbers of fluorescing cells were observed in preparations made on Day +4, although they were also evident on Days +2, 7, and 10.

Induction of Neutralizing Antibodies. As shown in Table III, cotton rats inoculated intranasally with approximately 100 CRID₅₀ of live Edmonston MV produced significant titers of virus-specific serum neutralizing antibodies to MV Edmonston. These antibodies were first detected in animals given a single injection of live MV on Day +10, reached maximum levels on Day 14 (GMPT = 6.3 log₂/0.05 ml), and remained near this level through Day 28. In contrast, cotton rats inoculated similarly with portions of the same virus pool inactivated with UV light did not produce detectable levels of virus-specific serum neutralizing antibodies to MV Edmonston.

Disease Symptoms. Animals were observed daily for up to 28 days after MV inoculation. No clinical signs of illness (lethargy, morbidity, diarrhea, weight loss, respiratory stress, mortality) were apparent in any animal inoculated in these experiments.

Discussion

The data presented in this report show that after intranasal inoculation of hispid cotton rats with a highly tissue-culture-adapted MV strain (Edmonston, ATCC VR24), there is an asymptomatic infection of the lungs with the development of scattered interstitial foci of virus and inflammation, and the production of significant levels of MV-specific antibodies. After clearance of the initial virus inocula, pulmonary virus replication was evident on Day +2, reached maximal levels on Day +4 (3.3–4.6 log₁₀/g lung in different experiments), and persisted for at least 7 days.

The presence of scattered, small foci of inflammation containing mixed leukocyte populations in lung sections stained with hematoxylin and eosin and similar diffuse areas of virus-specific fluorescence in frozen sections stained with an indirect fluorescent antibody

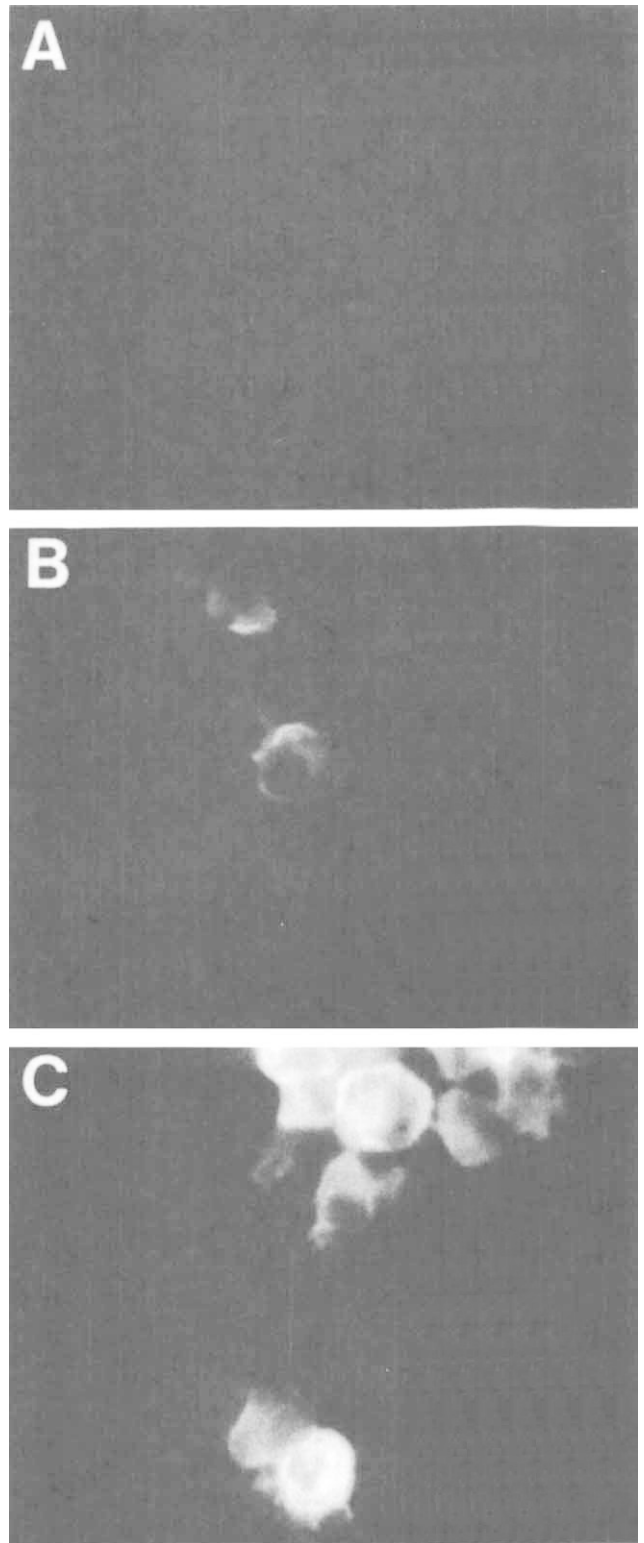


Figure 5. Photomicrographs of lung leukocytes pelleted onto glass microscope slides and stained using antiserum specific for PIV3 or MV and an indirect fluorescence procedure. (A) Lung leukocytes from a cotton rat 4 days after inoculation with MV stained with rabbit antiserum specific to PIV3 before the addition of fluorescein isothiocyanate-conjugated goat anti-rabbit serum. (B, C) Lung leukocytes from comparably inoculated cotton rats stained with rabbit antiserum specific to MV before the addition of fluorescein isothiocyanate-conjugated anti-rabbit serum. Fluorescence is predominantly cytoplasmic.

Table III. Comparison of the GMPT of Virus-Specific Neutralizing Antibody in Sera Collected at Different Times from Cotton Rats Inoculated with Live or Inactivated Edmonston MV^a

Virus inoculated	GMPT (log ₂) MV-specific neutralizing serum antibody on day						
	0	4	7	10	14	21	28
Measles							
Live	<2	<2	<2	3.0	6.3	6.2	6.0
Inactive	<2	<2	<2	<2	<2	<2	<2

^a Cotton rats were lightly anesthetized with ether and inoculated intranasally with approximately 100 ID₅₀ doses of live virus or the same virus after inactivation with UV light. At each test interval, the animals were bled from the retro-orbital sinus plexus. Serum from these bloods were processed, heat inactivated, and tested for virus-specific antibodies in 96-well microneutralization assays, as described in Materials and Methods. All values represent titers (log₂/0.05 ml).

technique for detecting MV-specific antigens both suggest limited, if any, MV replication in pulmonary parenchymal tissue. Instead, the presence of virus-specific fluorescence in many of the mononuclear cells in lung lavage suspensions from MV-infected cotton rats (>95% leukocytes containing both mono- and polymorphonuclear cells) after staining with the indirect fluorescent antibody procedure for virus-specific antigen and the recovery of a majority of viable virus from the cellular portion of lung lavage suspensions (again, >95% leukocytes) suggest that cotton rat leukocytes are the major target cell for MV replication after intranasal inoculation.

Several monkey species, particularly rhesus, appear to be highly susceptible to wild-type MV. After infection, unimmune animals may develop acute pneumonia with many of the characteristics seen in human MV disease (i.e., infection of the lung parenchyma and formation of syncytial giant cells, some of which contain both intranuclear and intracytoplasmic inclusions) (14–16, 26). Although the infection seen in cotton rats differs significantly from the MV infection seen in primates, the cotton rat model has several features that may make it utilizable for basic studies (e.g., MV-leukocyte interactions) and for testing anti-MV agents *in vivo*. These features include: (i) reproducibility (after intranasal inoculation, all inoculated hispid animals developed a pulmonary infection; Table I; 100% of the lung lavage cell fractions tested positive); (ii) the occurrence of infection in mature animals; and (iii) readily infected leukocytes (also a feature of primate measles disease). Indeed, initial studies using this model will investigate MV-leukocyte interactions and determine the effects of Edmonston MV on different immune functions. However, the model may have more potential in testing new MV vaccines or antiviral agents with potential anti-MV activity. Currently, other than primates, there is no *in vivo* model available for such testing.

Optimistically, it may be possible to develop the cotton rat model and make it a better measles paradigm. One of the three clinical MV isolates tested, AC705, grew well in the lungs of some of the cotton rats

inoculated with this virus. Thus, it may be possible to adapt this or other clinical MV isolates to produce clinical disease in cotton rats by serially passaging them in these animals, as has been done with some influenza virus strains in mice (27). It is also possible that other tissue-culture-adapted MV strains (e.g., the Enders vaccine strain) may grow better in cotton rats and induce a more severe disease than the ATCC Edmonston strain used in these experiments. This sanguinity is based on the supposition that the greater infectivity of the Edmonston MV strain for hispid cotton rats is related to alterations in its host range due to the multiple passages of this virus in tissue culture. It may be that a virus that has a greater or different passage history (e.g., Enders or another very high-passage MV strain) may grow better and be more virulent in cotton rats.

Maximal isolation of MV was obtained from samples obtained by transpleural lung lavage. As indicated in Figure 4, leukocytes comprise the great majority of cells obtained by transpleural lavage, and after centrifugation of lung lavage suspensions, these cells are concentrated. Another factor that may contribute to the apparent increased recovery of virus from lung lavage cellular fractions is that there is little obfuscation of tissue in the lower wells of virus assay plates when lavage suspension (in contrast to lung homogenates) is used in the titrations. Thus, it is easier to see and score positive wells when using lung lavage suspensions.

In summary, these findings, to the best of our knowledge, comprise the first report of MV replication in adult animals other than monkeys or humans after intranasal inoculation. Despite shortcomings, the MV-cotton rat model described may be useful for studying MV pathogenesis as well as for screening for and testing improved MV vaccines, immunomodulators, and/or compounds with potential anti-measles virus activity. Work is now in progress to test this potential and determine whether the model may be developed further.

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