Early Lymphoreticular Viral Tropism and Antigen Persistence
Tamiami Virus Infection in the Cotton Rat

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Tamiami virus was inoculated into its natural reservoir host, the cotton rat (Sigmodon hispidus),
and the course of infection was followed by sequential organ titrations, frozen-section immunofluorescence,
and light and electron microscopy. In animals infected at 2 days of age, there was an early
lymphoreticular tropism with peak concentrations of virus and viral antigen in lymph nodes, splenic
white pulp, thymus, and bone marrow at 16 days postinoculation. Megakaryocyte infection was early
and pronounced. Viral antigen concentration peaked in liver and salivary glands at day 30 and in kidney,
adrenal cortex, respiratory tract, and bladder epithelium at day 60—long after viral infectivity
in these organs had disappeared. Central nervous system infection was only modestly productive of
infectious virus, but viral antigen continued to increase in the brain until day 90 and then did not decline
throughout the 360-day study. Reticuloendothelial hyperplastic foci were found late in some target
organs, but there was never any histologic or ultrastructural evidence of cytonecrosis.

Older animals were virtually uninfecetable; therefore, this susceptibility of newborns and their slow
termination of infection represent the key to virus transmission and perpetuation in nature. These aspects
of viral natural history contribute to an understanding of human exposure to the pathogenic
arenaviruses which exist in similar rodent niches.

Additional key words: Arenavirus infection; Lymph node, spleen, bone marrow, and thymus infection;
Neurotropic infection; Reticuloendothelial hyperplasia.

An understanding of the natural history of arenaviruses in the mammalian species which serve as natural reservoir
hosts for human infection depends, more than with other viruses, upon an appreciation of varying balances
between viral replicative events and host defense mechanisms. Infection patterns in the cricetid rodent species
which perpetuate several arenaviruses in nature are asymptomatic, with unusually persistent viral carriage
and shedding (21, 23, 36). The basis for the chronicity in these reservoir species lies in the minimal direct cyto-
pathology caused by viral replication and the immunosuppressive and/or immunopathologic effects of the
lymphoreticular tissue infection. In such infections, all histologic changes which do appear are attributable
to immunopathologic host response, but the sites of damage are determined by the neuro- and epitheliotropisms
which are also characteristic of these viruses. The pathogenetic mechanisms in the most lethal arenavirus in-
fec tions, Bolivian hemorrhagic fever, Argentinian hemorrhagic fever, and Lassa fever of man, are also suspected
to be immunopathologic, but direct viral effects may contribute; these important questions concerning human
infection have not been resolved.

Much of the evidence regarding the pathogenesis of arenavirus infections has been derived from studies of
lymphocytic choriomeningitis (LCM) virus in mice (32). When attempts were made to expand studies in this
species to other arenaviruses such as Tacaribe and Tamiami viruses, a pronounced neurotropism was found
which evoked immunopathologic central nervous system disease, but which was unlike infection patterns in
feral rodent reservoir species, or in man or experimentally infected primates (3, 4, 10, 11, 41). Limited studies
of the hazardous arenaviruses have been undertaken in their natural reservoir species: Machupo virus (the agent
of Bolivian hemorrhagic fever) has been studied in Calomys callosus, and Lassa virus (the agent of Lassa
fever) is being studied in Mastomys natalensis (21, 22, 38). Because biohazards limit such studies, the present
experiments employing Tamiami virus, an agent not known to be pathogenic for man, were undertaken. In the
cotton rat (Sigmodon hispidus, family Cricetidae), the
only known natural host of this virus (20), the asymptomatic infection varied with host age at the time of exposure to virus. Organ titration and light, immunofluorescent, and electron microscopy were used to study the course of viral pathogenesis. To the extent that Tamiami virus lymphotropism affects chronicity of infection and viral shedding, it has become a requirement for the perpetuation of the virus in nature. The natural history of this virus in the cotton rat may be useful in predicting similar virus-host relationships involving arenaviruses which are human pathogens.

MATERIALS AND METHODS

Virus and Virus Titration

Tamiami virus, prototype strain W-10777, was originally isolated from cotton rats (Sigmodon hispidus) in South Florida by Calisher et al. (5). Stock virus had been passaged seven times in suckling mouse brain and had an infectivity titer of \(10^{7.5}\) LD\(_{50}\) or plaque-forming units (PFU) per gm. of brain tissue when titrated by intracerebral inoculation of newborn mice or by plaqueing in Vero cells (virtually identical titers were obtained in the two assays). All organ and tissue titrations were carried out by plaqueing in Vero cells by standard methods (25, 39).

Animals

Cotton rats (Sigmodon hispidus) were originally wild-trapped in Southeastern United States. No attempt was made to define subspecies (14). Animals were bred and raised in the facilities of the Veterinary Services Branch, Center for Disease Control, and were used after 5 to 20 laboratory generations. Repeated testing for intercurrent infection with LCM and Tacaribe viruses, the other two arenaviruses of North and Central America, was negative, and animals appeared healthy at all times.

Experimental Design

Groups of cotton rats were inoculated intraperitoneally with \(10^{6.9}\) LD\(_{50}\) (= PFU) of virus when they were 0 (= less than 18 hours), 2, 21, and 90 days of age. The younger animals were inoculated and held as litters with their uninoculated mothers. The most extensive study was carried out on the animals inoculated at 2 days of age; two of these were sacrificed on days 2, 5, 7, 9, 12, 16, 23, 30, 40, 60, 90, 120, 150, 211, 270, and 360 postinoculation. Specimens of spleen, thymus, lymph nodes (several mesenteric and inguinal nodes pooled), liver, kidney, submandibular salivary gland, and brain were collected for infectivity titration. Urine specimens were diluted 1:1 with tissue culture medium (10 per cent serum) for storage. These tissues and, in addition, muscle, heart, lung (with trachea attached), adrenal gland, pancreas, bladder, and spinal column or cord were prepared for histology and frozen-section immunofluorescence. Femoral bone marrow was collected for titration and for making thick smears for immunofluorescence. Attempts to collect urine were only successful in older animals (>10 days), so in younger animals bladders were titrated. Specimens of spleen, lymph nodes, salivary gland, and brain obtained on days 12, 16, 23, and 150 postinoculation were prepared for electron microscopy.

Pairs of animals inoculated on days 0, 21, and 90 of life were sacrificed on days 6, 12, 30, 60, and 90 postinoculation. Tissues were processed in the manner described for the animals inoculated at 2 days of age. Finally, litters of cotton rats, born to mothers which had been inoculated at 2 days of age, were also inoculated when 2 days old and sampled similarly. Uninoculated animals, including some littersmates kept together with inoculated siblings, were examined from each group.

Histology

Organs and tissues were fixed in 10 per cent buffered formalin and embedded in paraffin; sections were stained with hematoxylin and eosin.

Immunofluorescence

Organs and tissues were embedded in a polyethylene glycol compound (O.C.T., Ames Company, Elkhart, Indiana) and frozen on Dry Ice. Four-micrometer sections were dried, fixed in acetone at room temperature for 10 minutes, and treated with conjugate for 30 minutes. This conjugate has been described previously (41). Appropriate controls included inhibition tests, comparison tests with infected cell culture substrates, and age-matched uninoculated or sham-inoculated animal specimens treated in parallel; all controls were unremarkable.

Electron Microscopy

Tissues were cut into 1-cu.-mm. blocks and fixed in 2.5 per cent buffered glutaraldehyde for 2 hours at 4°C. Blocks were then treated with 1 per cent buffered osmium tetroxide, dehydrated in an ethanol series, and embedded in an Araldite-Epon mixture (28). Sections were stained with uranyl acetate and lead citrate and examined in a Philips EM-300 electron microscope.

Serology

Complement-fixing antibody was quantitated by the Laboratory Bureau Complement Fixation (LBCF) method in a microtiter system (6). Antigen was a sucrose-acetone-extracted infected mouse brain preparation (13), and control antibody was a hyperimmune mouse ascitic fluid. Neutralizing antibody was measured by plaque reduction in Vero cells using an 80 per cent reduction end point (39).

RESULTS

Tamiami virus caused no apparent disease in cotton rats varying in age from less than 18 hours to 90 days at the time of intraperitoneal inoculation. Cotton rats inoculated intraperitoneally at 2 days of age were chosen for the major part of this study. Variations observed in animals inoculated at other ages are considered in the following section. Differences between inoculated and control animals were not evident at necropsy except that the lymph nodes of infected animals were consistently larger than those of age-matched controls.

Organ and Tissue Infectivity Titrations

Spleen from one animal on day 2 postinoculation contained virus. By day 9, when viremia was initially...
detected, all lymphoreticular organs had rather high infectivity titers (Fig. 1). At this time the other major extraneural target organs—liver, kidney, and salivary glands—were infected; additional organs, which were tested did not have higher titers than blood from the same animals. All urine specimens were negative. There was a slight delay in viral invasion of brain, with one animal still negative on day 9, but by day 12 infection was well established. Peak virus titers were reached in all organs between days 12 and 16, and rates of decline varied thereafter. Ultimately the highest titers were obtained in salivary gland and liver (10^{7.5} and 10^{6.2} PFU per gm., respectively). The peak brain titer was 10^{5.1} PFU per gm., in sharp distinction to the exceptional yield from this organ in mice infected with Tamiami virus (41). In general, large amounts of virus were still present on day 30, but with the exception of one lymph node, all organs were sterile by day 40. The exceptional lymph node specimen from day 40 yielded 10^{4.0} PFU per gm., but by the time of the next harvest on day 60 and in all subsequent harvests up to the termination of the study on day 360, no infectious virus was found.

Complement-fixing antibody was first detected on day 16 and rose slowly thereafter to a plateau on day 60 (Fig. 2). Neutralizing antibody was initially found on day 23, coincident with the termination of viremia between days 16 and 23. The rapid rise in the titer of neutralizing antibody between days 23 and 30 made the high infectivity titers in target organs at this time all the more remarkable. The antibody titers persisted at high levels for the duration of the study, but neither complement-fixing nor neutralizing antibody was ever detected in mothers of inoculated litters or un inoculated control animals from the same feral source and laboratory colony.

**Lymphoreticular Organ Infection**

Tamiami virus antigen was initially detected on day 7 in the animals inoculated intraperitoneally at 2 days of age; however, immunofluorescence distribution patterns were better defined by day 9. At this time, antigen was localized in the cortex of lymph nodes and extended to the marginal sinus boundary (Fig. 3), in the white pulp of the spleen, with particular concentration in the marginal zone and periarteriolar sheath (Fig. 4), and throughout the cortex of the thymus (Fig. 5) (lymphoid histology terms from Weiss (40)). Taken together, the immunofluorescent patterns indicated an initial viral invasion of the reticular and epithelial-reticular cells of these organs and rapid subsequent infection of lymphoid cells. This was confirmed by day 16 when the same areas contained their highest concentrations of antigen. In lymph nodes and thymus, cortical immunofluorescence occurred as large foci involving all contiguous cells (Fig. 6). In the splenic white pulp this intense immunofluorescence often extended from the central arteriole to the marginal zone (Fig. 7). The antigen distribution in these cells was finely particulate throughout the cytoplasm. By day 23 the amount of antigen in these sites was greatly diminished; the thymic cortex was nearly negative; and in the splenic white pulp, antigen was diminished in lymphocytes, leaving the cytoreticulum as prominent as on day 9. In the spleen, the marginal zone reticulum was prominently involved at day 40 (Fig. 8), and this involvement declined until it was not seen after day 90. An exceptional observation in spleen specimens at days 40, 60, and 90 was infection of all of the layers, including the smooth muscle, of the central arteriole of white pulp; at the same time, similar sized arterioles were affected in the cortex of the kidney, but not elsewhere in these rats.

Viral antigen was also found in large cells, identified as macrophages, in the deep cortex and medulla of lymph nodes and thymus (dendritic cells) and in the red pulp of the spleen. Between days 9 and 30, a small but rather consistent number of these cells was involved in each animal, but only in splenic red pulp were they found in declining numbers on days 40, 60, and 90.

Germinal centers developed in lymph nodes and spleen by day 16 and reached striking size, number, and maturity by days 30 to 60 (Fig. 9). The grossly visible enlargement of lymph nodes of these young rats was caused by the number of these germinal centers with bulging of capsular surfaces. Germinal center margins were particularly well demarcated and there was macrophage (tingible body) activity throughout. In spleen, germinal center capping of white pulp was massive by 60 days, and in thymus no histologic changes were noted. This reactive state of lymph nodes and spleen was maintained throughout the 360 days of the study, and in the last specimens of lymph nodes (days 270 and 360) there was, in addition, a deep cortical hyperplasia with many large pale cells patchily intermixed with lymphoid cells (Fig. 10). No evidence of any viral cytopathology was ever seen in these organs by light microscopy. Uninfected, age-matched controls exhibited a few small and poorly defined germinal centers in a few nodes, but only after 120 days of age.

Electron microscopy of these lymphoreticular organs at times of highest infectivity titers and viral antigen concentration confirmed the lack of cytopathology, but few virus particles and inclusion bodies were found (29). These were associated with medium-sized lymphoblastoid cells as described previously in studies from this laboratory on Machupo virus in Calomys callosus rodents (31).

Two cell types were infected in bone marrow. First, megakaryocytes were infected very early so that by day 9 virtually all of these cells contained a very heavy concentration of immunofluorescent antigen distributed throughout the cytoplasm in a finely granular form. Identical overwhelming involvement of megakaryocytes in the red pulp of the spleen (Fig. 11) was observed at this time, and in both marrow and spleen this declined so that only approximately 10 per cent of these cells contained antigen on day 16 and none thereafter. Electron microscopy of splenic red pulp on day 9 indicated that megakaryocyte infection was productive (Fig. 12). Typical arenavirus particles with characteristic surface projections, envelope, and ribosomes contained in an otherwise unstructured interior (30) were located in platelet demarcation channels throughout these large cells (Fig. 13). There was no evidence of cytopathology or viral inclusion formation. As in the other lymphoreticulo-
Fig. 1. Growth of Tamiami virus in the principal target organs of cotton rats inoculated intraperitoneally at 2 days of age. Infectivity assays were performed on individual tissues by plaquing in Vero cells.
VIRAL LYMPHORETICULAR TROPISM

The over-all peak antigen concentration at 16 days in lymphoreticular organs contrasted sharply with much later peaks in other organs. In the liver, viral antigen was first detected on day 9 as extremely fine "dustlike" immunofluorescence spread thinly in the cytoplasm of a few individual hepatocytes. Fewer than 0.1 per cent of the cells was involved, and by days 16 and 23 the same fine immunofluorescent pattern was exhibited by a maximum of 1 and 3 per cent of the cells, respectively, often in small foci. At 30 days approximately 30 per cent of hepatocytes contained antigen in dustlike and large aggregate forms (Fig. 15); the distribution of infection was randomly focal. This 30-day peak diminished rapidly, so that by day 40 only a few hepatocytes fluoresced, and by day 60 none fluoresced. At these times macrophages at the edges of portal triads contained antigen, but from days 90 to 360 the liver was completely negative. No light microscopic changes were ever detected in the liver.

The same time course of antigen accumulation observed in liver was also noted in submandibular salivary gland. Although virus titers were high very early, antigen build-up in mucous acinar cells and ductal epithelium started on day 23 (Fig. 16), peaked on day 30, and declined to nil through days 40 to 90. By electron microscopy, a very productive infection was found. Virus particles were shed only from luminal (apical) plasma membranes of mucous acinar cells into the salivary space (Fig. 17). At days 60 and 90, but not afterward, most salivary gland specimens contained multiple, large perivascular foci of reticuloendothelial hyperplasia. Large numbers of macrophage-like cells invaded interacinar spaces (Fig. 18), but there was no associated parenchymal cytopathology, and when these cells were observed in frozen-section immunofluorescence, they did not contain viral antigen.

A still slower time course of antigen build-up was found in kidney, adrenal gland, respiratory tract, and bladder. In the kidney, immunofluorescence was first detected in calyx epithelium on day 23 and in very few tubules on day 30. On day 40, tubular involvement was still modest, but by day 60 a peak was reached with antigen detectable in half of the tubules. The pattern of antigen deposition changed from dustlike to aggregate and usually was similar in all of the cells of a transected tubule. Small amounts of antigen were localized in glomerular tufts at this time only. Tubular immunofluorescence declined by day 90, but in nearly all animals examined between days 90 and 270, a few tubules remained positive. Perivascular reticuloendothelial hyperplasia was pronounced in most animals between days 60 and 150 (Fig. 19), but as in the salivary gland, viral antigen was not found in mononuclear cells (Fig. 20).

There were no other histologic changes in the kidney. Adrenal cortical immunofluorescence started at day 30 and peaked on day 60. A massive involvement of the zona glomerulosa extended into the zona fasciculata (Fig. 21). Antigen became restricted to the zona glomerulosa at day 90 and persisted there until day 150. The respiratory tract (trachea, bronchi, bronchioles) was...
FIG. 3. Tamiami virus antigen in lymph node at 9 days postinoculation. At this time, infection was primarily in the reticulum and to a lesser extent in lymphoid cells. All micrographs are of tissues of cotton rats inoculated intraperitoneally at 2 days of age; immunofluorescence micrographs are of frozen sections stained directly with fluorescein isothiocyanate-conjugated anti-Tamiami virus globulin. ×520.

FIG. 4. Spleen at 9 days postinoculation. At this time viral antigen was concentrated in the reticulum of the marginal zone and periarteriolar sheath of the white pulp. ×360.

FIG. 5. Thymus at 9 days postinoculation. Early infection pattern indicated a primary involvement of the epithelial reticulum of the thymic cortex. ×520.

FIG. 6. Lymph node at 16 days postinoculation. At this peak time, massive accumulation of antigen in cortical lymphoid elements was superimposed upon the infected reticulum. ×360.
Fig. 7. Spleen at 16 days postinoculation. Intense immunofluorescence of virtually all lymphoid cells of the white pulp, from the central arteriole (right edge) to the marginal zone (left), reached a peak in spleen at this time. ×360.

Fig. 8. Spleen at 40 days postinoculation. Residual viral antigen was found in the reticulum of the marginal zone (upper right) and of the periarteriolar white pulp after clearance of lymphoid elements. ×520.

Fig. 9. Lymph node at 60 days postinoculation. Mature germinal centers were present from day 16 until the end of the experiment at day 360, whereas in uninfected animals germinal center development was rare. All paraffin sections were stained with hematoxylin and eosin. ×110.

Fig. 10. Lymph node deep cortex at 360 days postinoculation. Late in infection, in addition to the maintenance of germinal centers, there was deep cortical hyperplasia with patchily distributed large pale cells. ×300.
Fig. 11. Splenic megakaryocytes at 9 days postinoculation. At this time virtually every megakaryocyte in the spleen and bone marrow contained large amounts of viral antigen in granular form, but this diminished rapidly. ×520.

Fig. 12. Megakaryocyte at 9 days postinoculation. Typical Tamiami virus particles had surface projections, a unit-membrane envelope, and ribosomes within an otherwise unstructured interior. All thin sections for electron microscopy were stained with uranyl acetate and lead citrate. ×75,000.

Fig. 13. Megakaryocyte at 9 days postinoculation. This site of infection was particularly productive; large numbers of virus particles were present in platelet demarcation channels (arrows) but not in nearby extracellular spaces. ×18,000.
Fig. 14. Bone marrow at 40 days postinoculation. Antigen was present in a small proportion of medium-sized cells (tentatively identified as reticulum cells) in specimens from days 9 through 120. Composite; x520.

Fig. 15. Liver at 30 days postinoculation. At this time when there was a peak antigen load in this organ, large foci of hepatocytes contained dustlike and aggregate immunofluorescence. x520.

Fig. 16. Submandibular salivary gland at 23 days postinoculation. Ductal epithelium, as illustrated, and mucous acinar cells contained largest amounts of viral antigen after the time when organ infectivity titers reached a peak. x360.

Fig. 17. Submandibular salivary gland at 23 days postinoculation. Large numbers of virus particles accumulated in salivary secretion spaces, such as in this canaliculus, as a result of directional budding from plasma membranes of mucous acinar cells. x22,000.
FIG. 18. Submandibular salivary gland at 60 days postinoculation. Perivascular reticuloendothelial hyperplastic foci also resulted in infiltration of gland parenchyma, but there was no associated cytopathology. ×250.

FIG. 19. Kidney at 60 days postinoculation. Multiple foci of reticuloendothelial cells were prominent around small arteries in the cortex of this organ between days 60 and 120. ×270.

FIG. 20. Kidney at 60 days postinoculation. Viral antigen was present at this time in tubules and glomeruli, as illustrated, but never in the periarteriolar reticuloendothelial foci. ×360.

FIG. 21. Adrenal cortex at 60 days postinoculation. At this peak, infection was centered in the zona glomerulosa (bottom left) with variable extension into the zona fasciculata. ×520.
maximally infected on days 40 to 60, with more than half of the columnar-cuboidal epithelium containing antigen; less than 1 per cent of epithelial cells contained antigen by day 90, and the last immunofluorescent cells were seen on day 120. Subjacent structures and alveolar epithelium were negative. Despite the fact that bladder infectivity titers never exceeded the viremia level and that virus was not recovered from urine, the bladder transitional epithelium contained massive amounts of antigen on days 40 and 60. Immunofluorescence was limited to a few individual epithelial cells by 90 to 120 days and none were seen thereafter.

Viral antigen was not seen in other extraneural organs and tissues, even when searched for in frozen sections of whole animals; no other histopathologic changes were observed. Age-matched uninoculated animals never had reticuloendothelial hyperplasia in any organ or any other histopathologic lesion.

PERIPHERAL AND CENTRAL NERVOUS SYSTEM INFECTION

Peripheral nerves exhibited very little immunofluorescence at any time, but spinal and Gasserian ganglia progressed from negative at 9 days to a rather consistent involvement of up to one-third of their neurons on days 16 to 40 (Fig. 22). The antigen was usually dustlike and evenly distributed over the large cytoplasmic profiles of these neurons, but antigen aggregates also developed. This antigen carriage declined by day 60. Spinal cord infection was minimal and usually was limited to less than 5 per cent of grey matter neurons and very few tracts.

Brain infection, first detected by immunofluorescence on day 16, was extremely sparse until day 40, when an increasing number of neurons (up to 10 per cent) contained fine dustlike and aggregate antigen. Thereafter, an increase in the antigen mass and distribution occurred so that a peak was maintained from day 90 until the end of the experiment. Through most of this time, certain nuclei were consistently involved; the dentate gyrus (Fig. 23), the granule cells, and to a lesser extent the Purkinje's cells of the cerebellum (Fig. 24), the olfactory bulb (Fig. 25), the pyramidal cell layer of the hippocampus, and the large midbrain-thalamic nuclei. As in other organs, antigen was initially dustlike in form, but aggregation was more extreme in large neurons after 90 days than anywhere else in these animals. From day 150 onward, infected neurons often had blebs at their edges which were symmetrically shaped and contained viral antigen (Figs. 25 and 26). Consideration that these blebs were not artifacts was based upon repeated viral antigen specificity controls and the fact that they were "in" the tissue plane of frozen sections, not on top of sections. The nature of these blebs and their relation to neuronal integrity remain unknown.

Electron microscopy of the brain confirmed that virus particles were extremely sparse and that viral antigen accumulation coincided with inclusion body formation (Fig. 27). Typical arenavirus inclusions appeared most often near the periphery of neuronal cytoplasm; they were unbounded and uniquely constituted of amassed ribosome-like granules in a rather electron-dense matrix (Fig. 28). In addition, varying numbers of fine filaments were found in matrices or replacing matrices. The nature of these filaments is unknown. Viral inclusions were also found in oligodendroglia (Fig. 29) near infected neurons, and in the spinal nerve roots in Schwann's cells. At 150 days, similar inclusions were found in some cerebellar granule cells and oligodendroglia; as in earlier harvests, production of virus was not spatially associated with inclusions, and there was no cytopathology or inflammation. The only infection of brain surfaces was seen in one animal at day 360; several foci of perivascular cells in the meninges contained antigen.

INFECTION IN COTTON RATS INOCULATED AT OTHER AGES

Less extensive studies were made of animals inoculated before they were 18-hours-old and monitored for 90 days; similar viral development and clearance kinetics were noted in these animals. Viral antigen distribution and removal were also similar, and the only histologic differences from animals inoculated when 2-days-old were (1) single necrotic foci in the hippocampi of two of six animals examined 12 days after infection, and (2) the arrest of a few granule cells in the external germinal cell layer of the cerebellum (as described in mice by Friedman, Gilden, and Roosa (10)). When inoculated with the same dose and via the same route, 21- and 90-day-old rats yielded very little virus. In the group inoculated at 21 days of age, virus was isolated from only one animal; at 6 days this animal's spleen contained $10^{2.6}$; thymus, $10^{3.5}$; and urine, $10^{4.0}$ PFU per 0.1 gm. or ml. In the group inoculated at 90 days of age, virus ($10^{4.0}$ PFU per ml.) was isolated from only one urine specimen at day 12. Immunofluorescence and histology were uniformly negative in both of these groups. Complement-fixing antibody was formed more vigorously in the older animals (Fig. 30). The kinetics of antibody formation suggested that older animals may have had better early IgM responses, but limited serum volumes precluded testing.

Finally, some animals infected at 2 days of age were allowed to breed at 6 months of age. These mothers had high-titered neutralizing and complement-fixing antibodies, and presumably still had viral antigen in some organs. Newborns from these breedings did not contain infectious virus, and their organs were negative by frozen-section immunofluorescence. When these newborns were inoculated with virus (same dose and route) at 2 days of age and assayed by organ infectivity titration and immunofluorescence 12 days later, no evidence of infection was found.

DISCUSSION

The arenaviruses of public health importance, that is, Machupo, Junin, Lassa, and LCM viruses, each are perpetuated in nature via serial transmission among individuals and generations of particular rodent species (21). Human exposure and serious disease (hemorrhagic fever, menigitis, etc.) are incidental events in the natural history of these viruses, but our focus on this exposure is necessary for considering means of reducing human risk. Some characteristics of rodent infection
FIG. 22. Thoracic dorsal root ganglion at 23 days postinoculation. Dustlike and aggregate antigen was found in up to one-third of ganglionic neurons between days 16 and 40, and a lesser involvement persisted throughout the study. ×520.

FIG. 23. Brain at 90 days postinoculation. Dentate gyrus, as illustrated, was one of the brain nuclei consistently involved late in the infection course. ×520.

FIG. 24. Brain at 150 days postinoculation. In the cerebellum, granule cell layer infection was focal or massive, and Purkinje's cell infection was variable. ×360.

FIG. 25. Brain at 150 days postinfection. Olfactory bulb neurons had aggregate form antigen in their cytoplasm and antigen positive blebs at their edges. ×520.
FIG. 26. Brain at 150 days postinoculation; high magnification of two neurons in a midbrain nucleus. Specifically fluorescent marginal blebs of unknown nature were common from day 150 onward. ×1,300.

FIG. 27. Brain at 23 days postinoculation. Viral inclusions (arrows) were usually concentrated near the periphery of neuronal cytoplasm without any associated ultrastructural pathology. ×12,000.

FIG. 28. Brain at 23 days postinoculation; high magnification of one inclusion in Figure 27. The typical arenavirus inclusions were unbounded and made up of ribosomes and filaments embedded in an indistinct matrix. ×35,000.

FIG. 29. Brain at 23 days postinoculation. Oligodendroglia contained viral inclusions (arrows) but were otherwise indistinguishable from normal cells of the brain parenchyma. ×12,000
patterns suggest a tenuousness of viral survival in nature; for example, the extreme restriction of individual viruses to limited rodent species makes influences on such rodent populations a dominant regulative mechanism. In contrast, other infection pattern characteristics suggest that the viruses are long established and entrenched in their niches; for example, virus may be shed from infected rodents for long periods (e.g., Machupo virus in *Calomys callosus* (22)) or may be transmitted vertically with persistent tolerance (e.g., LCM virus in *Mus musculus* (18, 19)). Both mechanisms facilitate the perpetuation of virus during periods when new susceptible rodents are not available.

Tamiami virus is continually circulated among cotton rats (*Sigmodon hispidus*) in central and southern Florida (20). Interpretation of field data has suggested that transmission occurs via exposure to infected excretions. In light of the present study, viral pathogenesis and host response may greatly affect such transmission patterns. The long lasting humoral immunity evoked by infection in animals of all ages suggests difficulty in initiating a persistent tolerant cycle. Rats born of immune mothers were not infectable at birth and rats infected at 21 days of age or older never shed virus. In any case, the focal point for considering transmissibility is the newborn. In newborn cotton rats, transmission is favored by factors such as high susceptibility to small amounts of virus, high yield of infectious virus, slow rate of termination of infection and shedding, and lack of viral or crucial immunopathologic damage to infected organs. Susceptible newborns of one could become infected and mature to produce a passively protected generation which then would mature and give birth to a susceptible generation to complete the cycle. As an alternate to this two-generation cycle, microniche isolation and variation in degree of immunologic maturity of individuals at birth could also continually provide susceptibles. The high generation turnover rate and high population densities of rodent hosts would also contribute to the level of human exposure to pathogenic arenaviruses having such an infection-transmission cycle.

By any standard, the elimination of virus and viral antigen from the tissues of cotton rats was exceptionally slow. Cell mediated immune mechanisms are crucial factors in host reactivity to arenaviruses and lymphotropic infection resulting in specific antigen overload is an effective means of ablating or decreasing reactivity. The early filling of lymphoid and reticular cells of cotton rats with immunofluorescent antigen may be the morphologic equivalent of functional high zone tolerance (7). Mims (26) described similar antigen accumulation in many of the same organs of mice infected with LCM virus, and Hanaoka, Suzuki, and Hotchin (15) and Mims and Tosolini (27) showed that frank destruction of lymphoid tissues (particularly thymus-dependent lymphocytes) followed the lymphotropic LCM infection. Löhrer, Ehlerding, and Lehmann-Grube (24) also demonstrated lymphoreticular destruction in murine LCM infection, but this was not limited to thymus-dependent areas.

The kinetics of virus and viral antigen removal reflected the ultimate recovery of the reticuloendothelial system from these early effects and the variable effectiveness of the system in different organ systems. With the contribution of neutralizing antibody from day 23 onward, clearance of virus was complete. However, there did not appear to be any viral antigen removal from the central nervous system even at 360 days. These findings further indicated the relative sequestration of the brain parenchyma from reticuloendothelial processes, but the presence of antigen-containing blebs on infected neurons might be evidence of very late responsiveness or of a capping-shedding process. Whether this acts to the benefit or detriment of the host remains to be seen; in the rather analogous situation in subacute sclerosing panencephalitis of man, measles virus antigen and high titers of specific antibody are associated with immunopathologic processes and progressive disease (37).

T-cell-mediated immunopathology has been shown to have a central role in the pathogenesis of Tamiami virus meningoencephalitis in mice (10, 11). This infection is particularly neurotropic; lesions include peculiar focal neuronal necroses concentrated in cerebellum and hippocampus, meningitis, vasculitis (11, 41), and arrest of migration of cells of the external granular layer of the cerebellum (10, 12). Neonatal thymectomy is lifesaving and prevents development of all lesions including the cerebellar heterotopia (11). Other arenaviruses, likewise, are associated with immunopathologic disease, especially in species other than those infected naturally (2-4, 8, 21, 42). In such cases, there usually is highly restricted localization of viral infection and antigen expression so that there is a concentrated target for cell-mediated cytotoxicity. This focalization of antigen may occur in tissues which are necessary for life, in which case immunopathologic events may be lethal. This is the situation in LCM virus infection of the choroid plexus (7), and analogous localization must be considered in severe human arenavirus infections. In Tamiami virus-infected cotton rats, the damaging effects of cell-mediated immune activities may be blunted by the widespread distribution of antigen.

Inaccessibility of viral antigen to cytotoxic lympho-
cytes may be a partial explanation for the noncytopathic nature of Tamiami virus infection in cotton rats. The reciprocal relationship between infectious virus and immunofluorescent antigen in several organs, notably the brain, indicates a late modulation of the infection. Arenaviruses antigenically alter cell membranes, which then become an immunologic target. Restriction of target antigen to the interior of the cell late in infection, therefore, would prevent immunopathologic damage. Neutralizing antibody develops in close temporal relationship to the switch to a nonproductive infection and may be causally related. An analogous situation has been demonstrated in vitro with measles virus (35); when incubated in the presence of measles-specific antibody, persistently infected cell cultures accumulate viral antigen and cease production of infectious virus.

The common occurrence of reticuloendothelial hyperplastic foci in some of the infected organs of cotton rats suggested that this aspect of late responsiveness was overly exuberant. This kind of hyperplasia has been associated with response to massive amounts of antigens such as are present in chronic allogeneic disease (1). Such hyperplasias have been considered neoplastic and characterized as a reticulum cell sarcoma. When the antigenic stimulus is an infectious organism, reactive hyperplasia may also be so extensive and extreme that malignant lymphoma may be diagnosed histologically (9). For example, postvaccinial lymphadenitis has been repeatedly confused with varying categories of lymphoid malignancies (16), and terms otherwise reserved for describing neoplasias have been used to represent interstitial mononuclear cell infiltrates in kidneys, liver, and lungs of mice persistently infected with LCM virus (18, 34). In LCM virus infection, a direct correlation between the reticuloendothelial hyperactivity and “late disease” (18, 32) or wasting (17) has been suggested; this correlation is weakened by the lack of detrimental effects of the same remarkable hyperplasia in Tamiami virus-infected cotton rats.

In experimental arenavirus studies there have been no evidences of lesions like those which led to the human disease” (18, 33) or wasting (17) has been suggested; this correlation is weakened by the lack of detrimental effects of the same remarkable hyperplasia in Tamiami virus-infected cotton rats.

Tamiami virus infection in the cotton rat may represent a step toward persistent tolerant infection. Vertical transmission, as the ultimate characteristic of persistent tolerance, may only be possible after an effective level of immune indifference has been achieved. Initiation of a persistent tolerant infection cycle, which would require precise timing of antigen exposure and immune ontogeny, may be the most difficult hurdle; this appears not to have been achieved in the Tamiami virus-cotton rat pairing. It may be further hypothesized that each arenavirus-rodent host pairing represents a different stage in a progression toward a persistent tolerant state, such as that which may be exhibited by LCM virus in Mus musculus. Variations in all arenavirus pathogenetic patterns must be defined precisely in order to test these hypotheses. In any case, data on these variations may be of value in predicting viral shedding and the potential for human exposure to the pathogenic arenaviruses.

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