

Simian Varicella Virus (Delta Herpesvirus) Infection of Patas Monkeys Leading to Pneumonia and Encephalitis^{1,2} (41342)

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Abstract. Patas monkeys were infected with Delta herpesvirus (DHV) to determine the pathogenicity of this virus in the lung and brain in order to assess the suitability of this model for severe complications in human VZV infection. Clinically, pneumonia and encephalitis occurred following intracerebral inoculation. Intratracheal or intravascular inoculation of DHV produced pneumonia but not encephalitis. Prominent microscopic lesions included diffuse interstitial pneumonia and focal hemorrhagic encephalitis. Typical herpesvirus intranuclear inclusion bodies were noted in lung parenchymal cells and in glial cells of the brain. DHV was isolated from peripheral blood leukocytes, spleen, lymph nodes, lung, and from various sites in the peripheral and central nervous system. Fluorescent antibody staining confirmed the identity of DHV recovered from monkey tissue. This study provides further evidence that the patas–DHV model has potential for predicting drug and/or immunotherapy regimens which may be useful in treating pulmonary and neurological complications of VZV infection.

Simian varicella is a naturally occurring exanthematous disease of non-human primates. Epizootics have been reported in patas (*Erythrocebus patas*), macaque (*Macaca nemestrina*, *M. fuscata*, *M. fascicularis*), and vervet (*Cercopithecus aethiops*) monkeys (1–4). The designated simian varicella virus isolates and their respective hosts are Delta herpesvirus (DHV)–patas, Medical Lake macaque (MLM)–macaque, and Liverpool vervet virus (LVV)–vervet. Simian varicella viruses, as with human varicella-zoster virus (VZV), are highly cell-associated host-specific herpesviruses. Serological studies indicate that simian varicella viruses and VZV are antigenically very closely related (5, 6).

Many of the clinical, pathologic, and virologic features of DHV infection in patas

monkeys closely parallel those observed in varicella (chicken pox) in humans (1, 7–9). For this reason, and the fact that an animal host permissive for VZV-induced pathology is lacking, investigators have previously noted that experimental DHV infection in the patas monkey is a good model for human varicella (8–10). Recently, Myers *et al.* (11) demonstrated that the guinea pig is susceptible to VZV infection although no clinical signs or pathology were observed.

We present in this report additional pathologic and virologic observations concerning experimental DHV infection in patas monkeys. We demonstrate for the first time that DHV has neuropathogenic capability and can readily produce encephalitis concomitant with pneumonia in patas monkeys. These results indicate that experimental DHV infection in patas monkeys is potentially an important animal model for studying the pathogenesis of life-threatening complications such as those that can occur in neonates and immunocompromised cancer and transplant patients with VZV infection.

Materials and Methods. *Cell culture.* Human diploid embryo fibroblast (HDEF)

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and Vero cells were grown at 37° in plastic flasks using Eagles' MEM (EMEM) supplemented with 5–10% fetal bovine serum (FBS), penicillin–streptomycin, 0.75% NaHCO₃, and 2 mM glutamine. Cell cultures and virus stocks were routinely examined for mycoplasma by fluorescent staining with 4',6'-diamidino-2-phenyl-indole (12).

Virus propagation. Two DHV isolates were used in this study. DHV, isolate 592-S, was obtained at tissue culture passage 30 from Dr. Ambhan Felsenfeld (Covington, La.) via Dr. Fred Rapp (Hershey, Pa.). Another DHV isolate, 355, was recovered from a monkey (patas 355) that contracted simian varicella at the National Institutes of Health. Patas 355 was housed with a patas monkey which was previously infected with DHV and subsequently shed reactivated DHV. Both DHV isolates were propagated in Vero cells and VZV in HDEF cells by mixing infected and uninfected cells as previously described (13). Alternatively, more sparsely infected Vero cell cultures were passed at 1:2 split ratios several times until DHV cytopathic effects (CPE) involved 50–100% of the cell monolayer.

Patas monkey inoculations. Laboratory-reared juvenile patas monkeys 8–14 months of age were used in this study. The monkeys were prescreened for presence of antibody to DHV by enzyme-linked immunosorbent assay (ELISA) to insure that only seronegative animals were used. Monkeys were infected in the order listed in Table I beginning with patas 697. Patas 685 was the only monkey that received DHV isolate, 355.

Just prior to inoculation of monkeys with DHV, infected cells were harvested by trypsinization, washed two times in EMEM containing 10% FBS, and resuspended to an appropriate volume in the same media without FBS. The inoculum was titrated retrospectively on Vero cells by infectious center assay as previously described (14). The inoculations were administered by either of several routes, viz., subcutaneously (SC), intradermally (ID) at three sites on the abdomen, intratracheally (IT), intravascularly (IV, carotid artery), or intracerebrally (IC) in each hemisphere (Table

I). Following inoculation each monkey was housed in a separate isolation chamber to prevent cross-infection. Blood samples from each monkey were taken at 3, 5, 7, and 10 days after inoculation in order to perform SGPT, SGOT, alkaline phosphatase, BUN, and CBC determinations.

Serology. The ELISA procedure utilized for screening patas sera was performed essentially as described by Castellano *et al.* (5). DHV ELISA antigen was prepared from Vero cells having 75–100% DHV CPE. Control antigen was prepared in the same manner from uninfected Vero cells. Rabbit anti-human γ globulin alkaline phosphatase conjugate (M.A. Bioproducts) was found to be quite satisfactory for detecting antibody to DHV in patas sera.

The indirect fluorescent antibody (FA) procedure utilizing goat anti-human IgG conjugate (Litton Bionetics) was carried out to confirm the identity of reisolated DHV. The reactivity of convalescent serum from patas monkey 697 was tested against DHV- and VZV-infected cells.

Recovery of DHV from monkeys. Patas monkey tissues were removed, chilled on ice, and processed for cocultivation within 2–4 hr. The tissues were processed by either of several methods for DHV isolation. Brain tissue was homogenized in EMEM with 10% FBS by passage through successively smaller gauges of syringe needles. Cells were dispersed from lymph nodes and spleens by teasing with forceps. All other tissues were finely minced with scissors. Peripheral blood leukocytes (PBL) isolated by Ficoll–Hypaque gradients were obtained from blood taken at various times after infection. Cerebral spinal fluid (CSF), throat swabs, and sera were centrifuged at 1500 rpm for 10 min in order to cocultivate DHV present in cell-free supernatants and/or cell-associated pellets. Tissue specimens were mixed with approximately $3\text{--}5 \times 10^6$ Vero cells and centrifuged at 1500 rpm for 10 min. After 30 min at room temperature the cell pellets were seeded in 75-cm² flasks. Cell cultures were passaged 48 hr postseed at split ratios of 1:2 to 1:3. A virus isolation attempt was considered negative if there was an absence of DHV CPE after three serial passages of

the cocultivated tissue in no less than 18 days. Previous experience indicated that DHV was most frequently isolated following only one but never after three passages of the cells. In order to provide an estimate of the relative quantity of DHV-infected cells present in a given tissue, the CPE was scored on a $\pm$ to 4+ scale as determined by microscopic observation after one passage of the cocultivated tissue and cells.

Histology. Monkey tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned in 5–6 μ m, and stained with hematoxylin and eosin.

Results. Clinical signs. The clinical signs which accompanied experimental simian varicella in this study are summarized along with virus inoculum doses and route(s) of inoculation for each monkey in Table I.

Clinical infection as indicated by anorexia and fever (1–3°F above normal) was observed by Day 3 with the exception of patas 685 where these signs were not observed until Day 7. Axillary and/or inguinal lymphadenosis occurred in five of the monkeys 3–4 days after infection and by 9 days for patas 685. A mild exanthem consisting of small papules at the inoculation sites occurred only in the two monkeys inoculated ID. A generalized widespread papular rash occurred only in patas 685 at Day 8 after infection. The rash evolved to a vesicular exanthem of the face, trunk, and limbs by Day 9. Patas 695 and 185 developed pneumonia which was evident from respiratory distress at Day 4. Pneumonia was not observed in patas 685 until Day 8 which is consistent with the later onset of other clinical signs. Following IC inoculation, lethargy, convulsions, and encephalitis were apparent by Day 4 in patas 733 and 736 and by Day 8 in patas 685. Clinical blood tests indicated that SGOT levels were elevated in all six monkeys while SGPT, ALK, BUN, and CBC values were within normal limits.

Pathological studies. Patas 697 was the first monkey inoculated in this study. As illustrated in Table I, this monkey had a mild infection without sequelae. For this reason, the animal was not killed for pathological study. DHV given to this monkey was at cell culture passage 42

whereas the other five monkeys received low cell culture passaged (Table I) DHV which was isolated from the previously infected monkey.

Patas 695 was inoculated in a manner similar to patas 697 except that the inoculum contained a low cell culture passage of DHV isolated from PBL of patas 697. In marked contrast to patas 697, monkey 695 showed severe clinical illness with respiratory distress evident on Day 4. This monkey died with pneumonia on Day 5. Gross lesions at necropsy included small papules at the ID inoculation sites and axillary and mesenteric lymphadenosis. The left lobe of the lungs was edematous and consolidated; the right diaphragmatic lobe was congested and the apical lobe appeared normal. The remaining major organs had no discernable gross lesions and the brain was not examined. Microscopic lesions in the lungs of patas 695 as well as patas 185, 736, and 685 demonstrated, collectively, the spectrum of pathology associated with viral pneumonia. The lesions, as shown in Fig. 1, included the following: (i) diffuse edema in bronchi and alveoli, (ii) mononuclear cell peribronchiolar infiltrate, (iii) interstitial alveolar septal thickening, (iv) focal parenchymal necrosis, and (v) eosinophilic intranuclear inclusion bodies in interstitial parenchymal cells. Other noted microscopic lesions in tissues of patas 695 were diffuse cloudy swelling in the liver with mononuclear cell cuffing around portal triads; a few hepatocytes contained inclusion bodies. The lymph nodes were hyperplastic with enlarged germinal centers. No lesions were observed in the spleen, pancreas, thymus, adrenal gland, or kidney.

Patas 185 was inoculated by IV route in order to determine if DHV could cross the blood–brain barrier and cause a CNS infection. As with patas 695, this monkey developed severe respiratory distress and pneumonia and was killed when moribund on Day 5. At postmortem the only gross lesion in the brain was leptomeningeal hemorrhage in the cerebrum. Axillary and mesenteric lymphadenosis was noted and edematous hemorrhagic pneumonia was apparent in the lungs. In the spleen, the red pulp was congested and a few cells in this

area contained intranuclear inclusion bodies. No microscopic lesions were observed in cerebrum, cerebellum, spinal cord, liver, and kidney.

Patas 733 as well as 736 and 685 was inoculated IC to determine whether or not DHV could induce CNS lesions similar to those observed with VZV CNS involvement in humans. Following the development of anorexia and fever, patas 733 became lethargic and convulsions began on Day 3 after infection. Respiratory distress was not apparent and the monkey became comatose and was killed when moribund on Day 5. At postmortem it was observed that IC inoculation sites on each frontal lobe were hemorrhagic as was the meninges. Lymph nodes were enlarged but no other gross lesions were observed. As depicted in Fig. 2, microscopic lesions in the cerebrum were leptomeningitis and focal hemorrhagic encephalitis with necrosis, gliosis, perivascular cuffing, and eosinophilic inclusion bodies in glial cells in proximity of the needle tract. Cerebellar leptomeningitis was noted with meningeal vessel dilatation and mononuclear cell infiltrate between the arachnoid space and pia mater. No microscopic lesions were observed in the lung, spinal cord, spleen, liver, or kidney.

The clinical features resulting from DHV infection in patas 736 were essentially the same as patas 733 (Table I). The gross pathology consisted of meningeal hemorrhage in the cerebrum. The single observed microscopic lesion in the brain was cerebral hemorrhagic leptomeningitis. Lesions in the liver were similar to those described for patas 695. No lesions were found in the cerebellum, spinal cord, spleen, kidney, pancreas, or lymph nodes.

Patas 685, as noted previously, was inoculated IC with a different DHV isolate than the other five monkeys. In this monkey convulsions began on Day 8; a papular rash appeared on the face, chest, abdomen, and limbs. Patas 685 became moribund and was killed on Day 10. Gross lesions at postmortem were widespread and included vesicular exanthem, hemorrhagic leptomeningitis of the cerebrum, splenohepatomegaly, multiple hemorrhagic foci of the lungs, and pronounced lymphade-

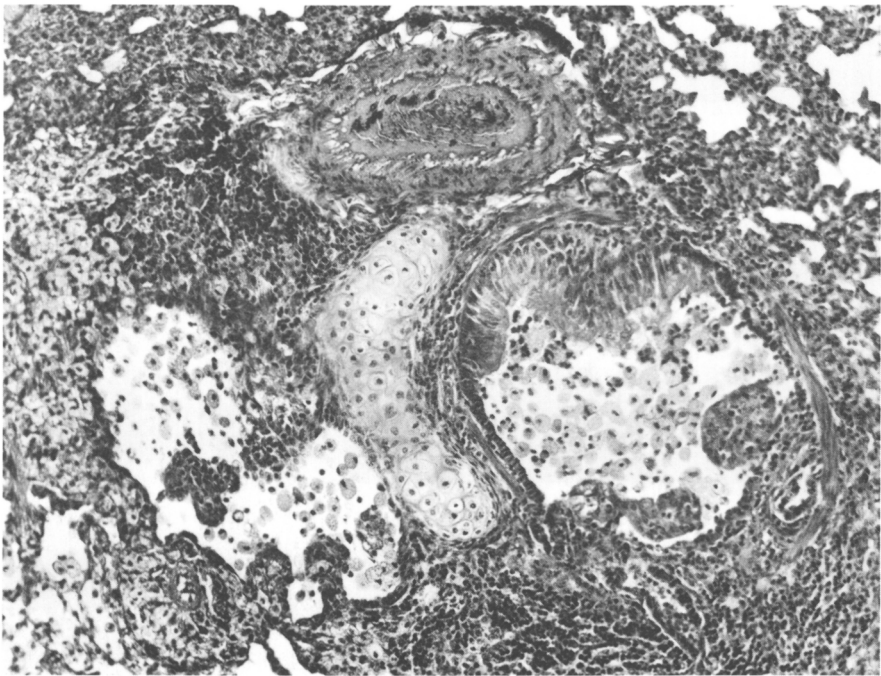
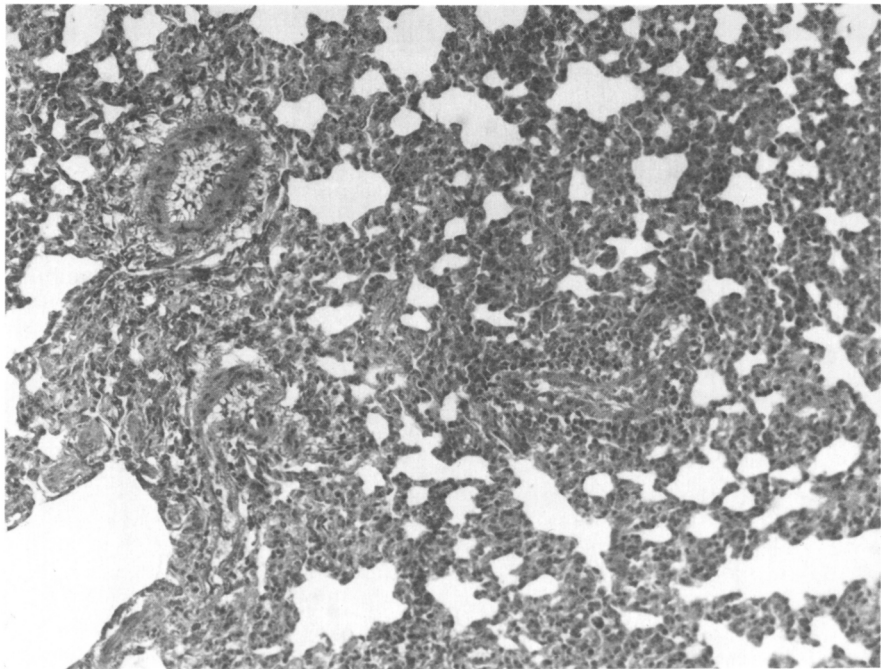
nosis. Microscopic lesions and involved tissues included the following: (i) skin—many vesicles approximately 3 mm in diameter containing clear serous fluid and many eosinophilic inclusion bodies in epithelial cells at the periphery of the vesicles, (ii) cerebrum—severe leptomeningitis, widespread hemorrhages, perivascular infiltrates, necrosis, gliosis, hemorrhage surrounding the needle tract, and eosinophilic intranuclear inclusion bodies in many glial and neural cells, (iii) cerebellum—meningitis and focal hemorrhages in the granular layer, (iv) spinal cord—hemorrhagic myelitis with focal gliosis (Fig. 3), (v) spleen—focal necrosis, (vi) liver—focal necrosis, and (vii) pancreas—severe pancreatitis with foci of necrosis and interstitial hemorrhage. No lesions were found in kidney, adrenal glands, thymus, or testes. The widespread vesicular exanthem, severity of clinical signs, and extent of pathology in this monkey were analogous to those characteristic of generalized varicella in humans (16, 17).

In summary, the above findings demonstrate that appropriate inoculation of DHV can readily produce systemic infection with severe interstitial pneumonia. DHV is neuropathogenic when inoculated IC. The comparison of CNS lesions associated with VZV infection in humans with DHV-induced CNS lesions in the patas monkey is shown in Table II.

Virological findings. Vero cells infected with DHV after cocultivation with infected monkey tissue formed focal clusters of rounded cells 2–3 days after cell passage 24–48 hr postinfection and within 3 more days these clusters developed into plaques (Fig. 4). This result is characteristic of DHV cytopathology (7).

The kinetics of DHV isolation from throat swabs, serum, PBL, and axillary or inguinal lymph nodes of patas 697 are illustrated in Table III. Based on these findings, the same specimens from other monkeys, when collected for DHV isolations, were taken at these times. DHV recovery from a Day 5 throat swab was the only positive throat swab isolate obtained during this study.

The results of DHV isolation from the



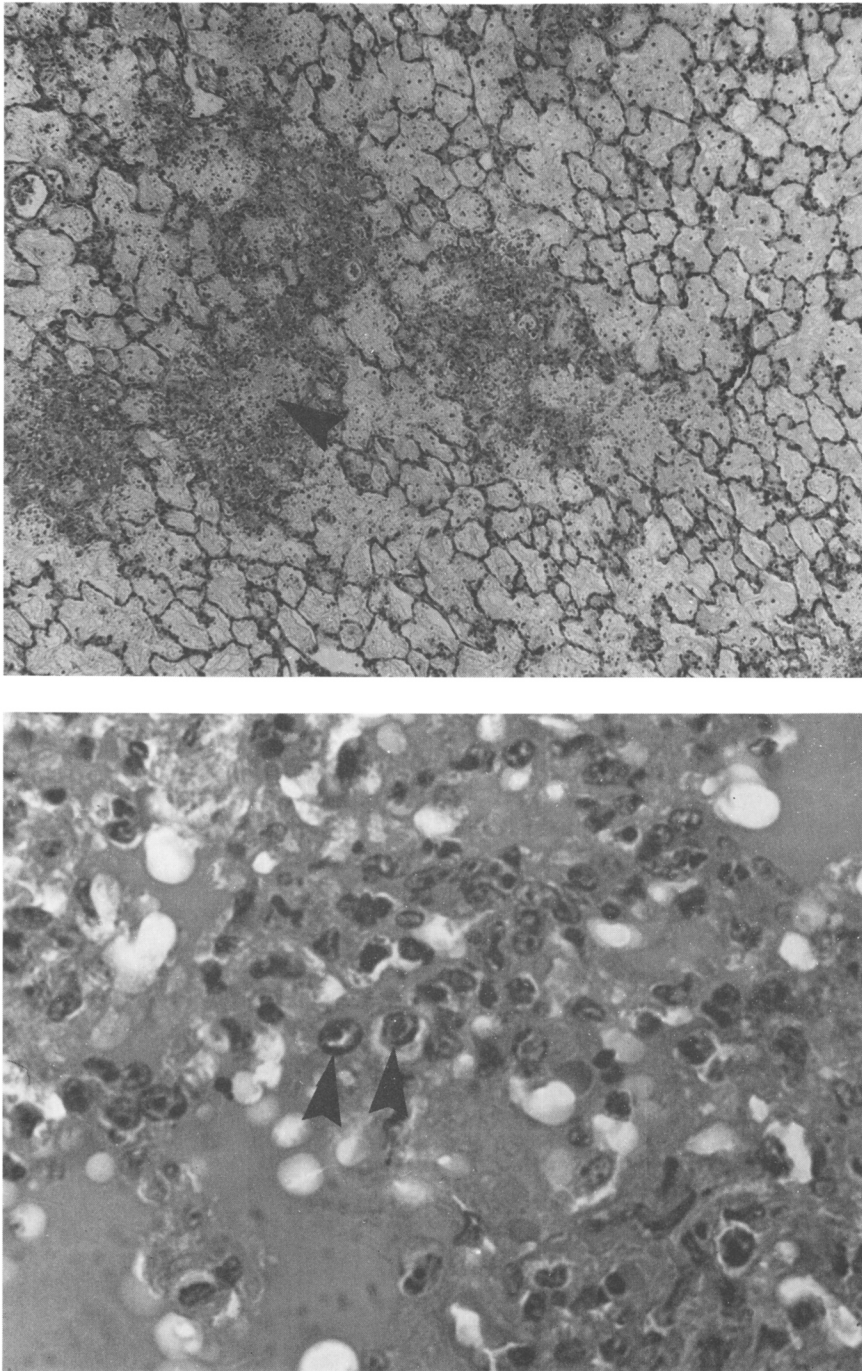
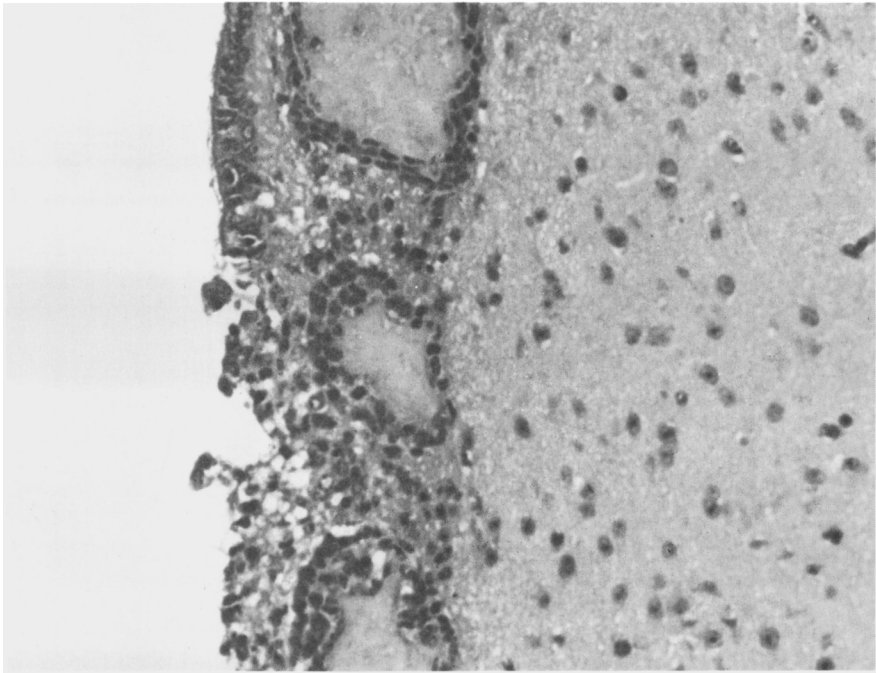
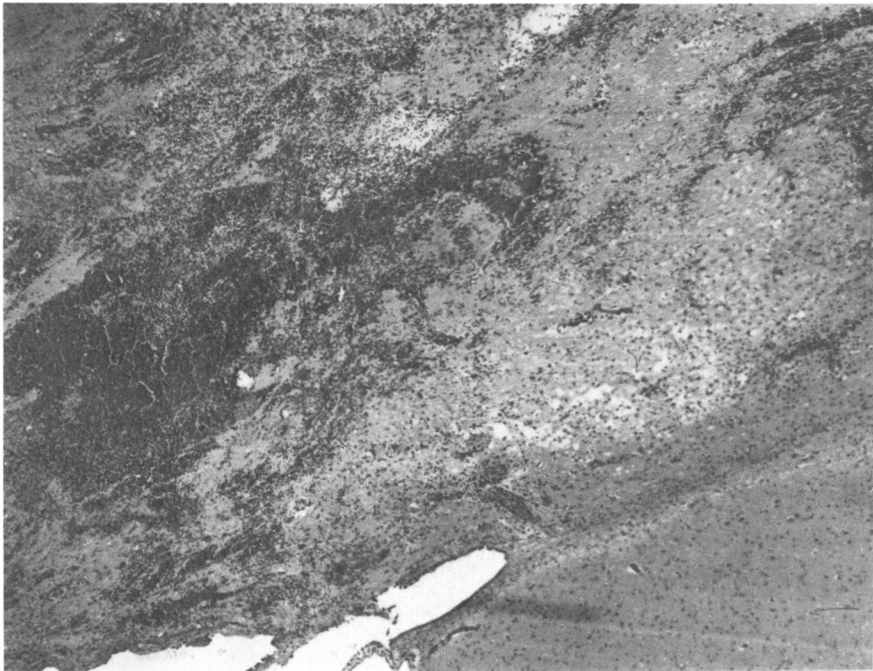


FIG. 1. Delta herpesvirus induced pneumonia in the patas monkey. Upper left: Peribronchial pneumonia with infiltration of lamina propria and tissue space with lymphocytes ($\times 100$). Upper right: Interstitial pneumonia with diffuse alveolar septal thickening ($\times 100$). Lower left: Intranuclear inclusion bodies (arrows) in parenchymal lung cells. Also, edema and fibrinous exudate are present in intercellular spaces ($\times 400$). Lower right: Focal necrosis (arrow) with diffuse fibrinous pneumonia ($\times 400$). All sections were stained with hematoxylin and eosin.



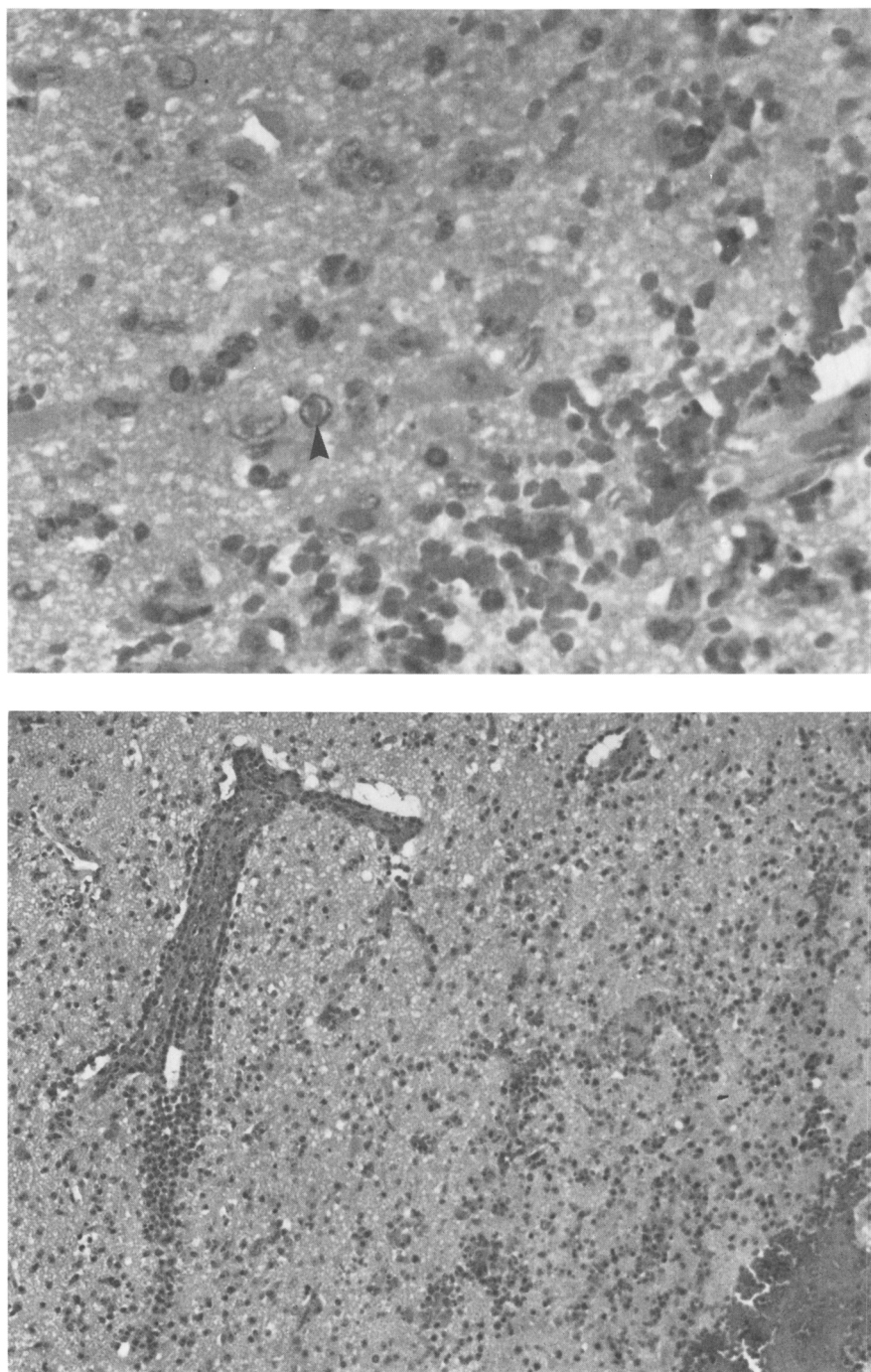


FIG. 2. Cerebral lesions associated with Delta herpesvirus infection in the brain of a patas monkey. Upper left: Marked thickening of the leptomeninges. Serous fluid and perivascular cuffing are evident ($\times 250$). Upper right: Focal hemorrhagic encephalitis; necrosis is evident at the periphery of the lesion ($\times 25$). Lower left: Mononuclear cell perivascular cuffing ($\times 400$). Lower right: Hemorrhagic encephalitis with gliosis. An intranuclear inclusion body is also shown (arrow) ($\times 400$). All sections stained with hematoxylin and eosin.

TABLE I. CLINICAL SIGNS OF SIMIAN VARICELLA FOLLOWING THE INOCULATION OF PATAS MONKEYS WITH DHV

Patas Monkey	Route of infection	Dose (ml)	Infectious cell titer (ml)	DHV origin	Clinical signs
697	Intratracheal Subcutaneous Intradermal	2.0 2.0 0.6	1.0×10^5	Cell culture passage 42	Anorexia, fever, papules, axillary lymphadenosis
695	Intratracheal Subcutaneous Intradermal	2.0 2.0 0.6	4.5×10^5	697 PBL isolate; passage 3 in Vero cells	Anorexia, fever, papules pneumonia, axillary lymphadenosis
185	Intradermal Intravascular	0.6 5.0	6.0×10^5	695 lung isolate; passage 8 in Vero cells	Anorexia, fever, pneumonia axillary, and inguinal lymphadenosis
733	Intracerebral	0.25 at two sites	2.6×10^5	185 lung isolate; passage 3 in Vero cells	Anorexia, fever, axillary and inguinal lymphadenosis, lethargy, convulsions, encephalitis
736	Intracerebral	0.25 at two sites	4.4×10^5	733 spleen isolate; passage 3 in Vero cells	Anorexia, fever, pneumonia, lethargy, convulsions, encephalitis, comatose
685	Intracerebral	0.25 at two sites	1.0×10^6	355 PBL isolate (NIH); passage 4 in Vero cells	Anorexia, fever, pneumonia, axillary and inguinal lymphadenosis, generalized papular rash and vesicles, convulsions, encephalitis

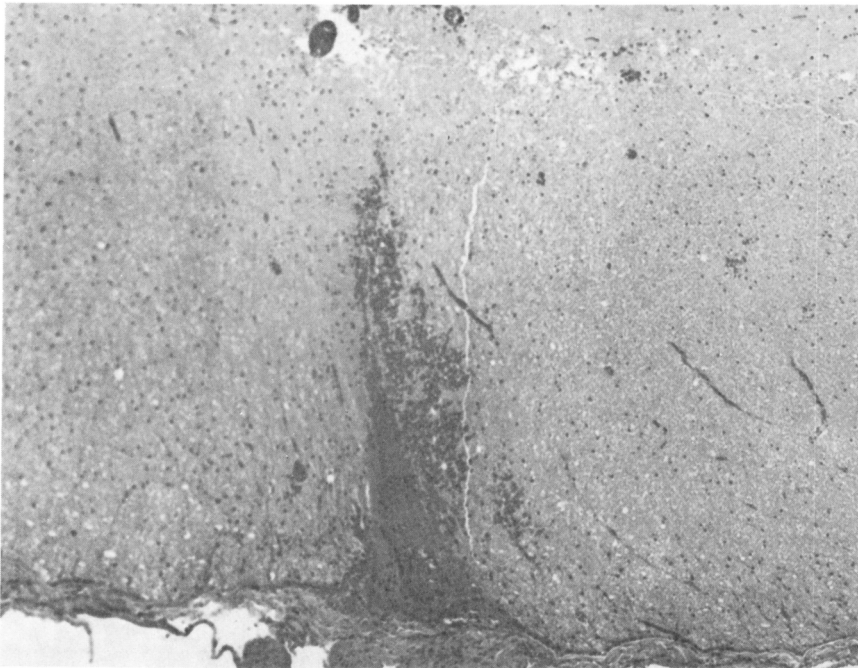


FIG. 3. Spinal cord pathology associated with Delta herpesvirus infection in the central nervous system. Severe hemorrhagic myelitis is present in the ventral aspect of the cord ($\times 40$). Hematoxylin and eosin staining.

various tissues of all other monkeys are listed in Table IV. DHV is highly cell associated; therefore, the extent of DHV-specific CPE in cells cocultivated with various tissues was proportional to the amount of input virus and was scored on a relative scale. Cell-associated but not cell-free viremia was readily demonstrated

since DHV was recovered from PBL but not the sera of the monkeys. Isolation of DHV from lymph nodes and a wide variety of tissues after nonparenteral inoculation was indicative of the systemic spread of virus (Table IV).

An interesting result was the fact that DHV was recovered from the cerebrum and

TABLE II. NEUROPATHOLOGY ASSOCIATED WITH HUMAN AND SIMIAN VARICELLA VIRUS (DHV) INFECTION IN CENTRAL NERVOUS SYSTEM

Lesion	Virus	
	VZV	DHV
1. Encephalomyelitis	+	+
2. Focal hemorrhagic necrosis (cerebrum)	+	+
3. Gliosis (cerebrum and spinal cord)	+	+
4. Meningoencephalitis	+	+
5. Leptomeningeal hemorrhage	+	+
6. Leptomeningitis	+	+
7. Glial cell intranuclear inclusion bodies	+	+
8. Perivascular inflammatory infiltrates	+	+
9. Segmental demyelination	+	-
10. Spinal cord necrosis	+	-
11. Virus isolation from CSF	+	+

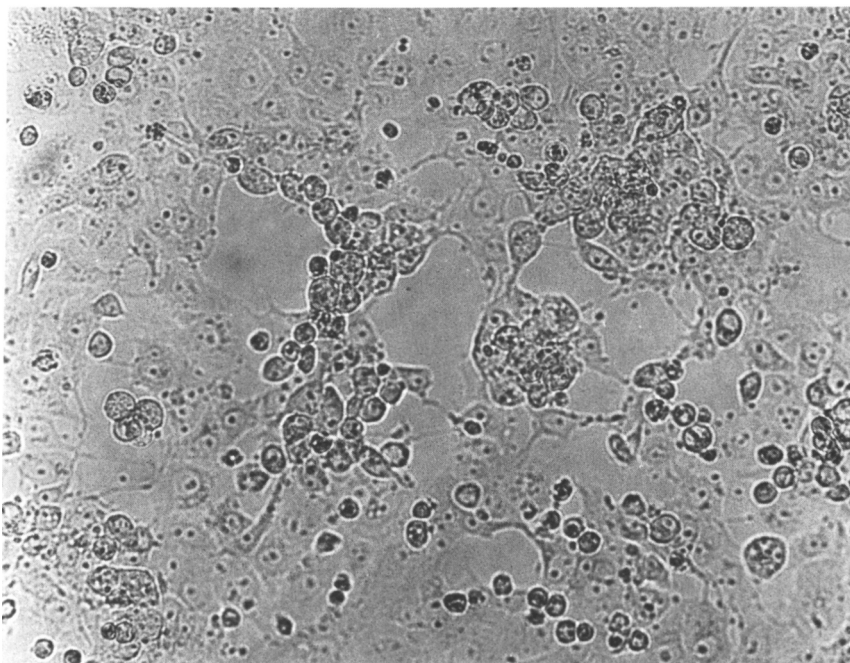


FIG. 4. Delta herpesvirus infection in Vero cells following cocultivation of infected patas monkey tissue. Clusters of round infected cells and plaque formation are evident ($\times 200$).

spinal cord of patas 185 indicating the spread of virus to the CNS following intravascular (carotid artery) inoculation. DHV was recovered from various sites in the central and peripheral nervous system after IC inoculation of patas 733, 736, and 685 (Table IV) viz., cerebrum, cerebellum, choroid plexus of the third ventricle, optic nerve, trigeminal ganglion, and spinal cord. DHV was not recovered from the dorsal root ganglion of patas 736 and 685. Although DHV was readily recovered from lymphoid tissues of patas 685, no virus was recovered from the thymus. Only cell-associated DHV was recovered from the CSF of patas 733, 736, and 685.

Immunofluorescence. At periodic inter-

vals during the course of this study, FA staining of cell cultures infected with original input (592-S) and reisolated DHV was carried out. Convalescent but not prebleed serum from patas 697 was reactive to DHV-infected Vero as well as VZV-infected HDEF cells. Conversely convalescent serum from an individual with herpes zoster was reactive to DHV and VZV-infected cells. Both nuclear and cytoplasmic FA staining were prominent. Uninfected cells did not exhibit specific fluorescence when reacted with the same sera. These FA staining results confirmed that the biologic properties of reisolated virus in cell culture were those of DHV.

Discussion. We have shown in this study

TABLE III. KINETICS OF DHV ISOLATION FROM PATAS MONKEY 697

Specimen	Day postinoculation			
	3	5	7	10
Throat swab	Neg	+	Neg	Neg
Serum	Neg	Neg	Neg	Neg
Peripheral blood leukocytes	+	+	+	Neg
Lymph node	ND ^a	+	+	ND

^a Not done.

TABLE IV. DHV ISOLATION FROM PATAS MONKEYS WHICH DEVELOPED PNEUMONIA AND/OR ENCEPHALITIS

Tissue or specimen	Patas monkey				
	736 (Day 6 PI) ^a	733 (Day 5 PI)	685 (Day 8 or 10 PI)	185 (Day 5 PI)	695 (Day 5 PI)
Throat swab	Neg	—	Neg	Neg	Neg
Serum	Neg	Neg	Neg	—	—
Peripheral blood leukocytes	3+ ^b	1+	—	1+	3+
Lymph node	1+	2+	1+	1+	1+
Lung	2+	3+	1+	3+	4+
Spleen	2+	3+	Neg	2+	4+
Adrenal	2+	—	—	—	—
Liver	Neg	—	Neg	±	—
Pancreas	Neg	—	—	—	—
Kidney	Neg	—	—	—	—
Cerebrum	2+	1+	4+	±	—
Choroid plexus (lateral ventricle)	—	—	3+	—	—
Cerebrum in proximity of third ventricle	—	1+	—	Neg	—
Cerebellum	1+	1+	±	Neg	—
Optic nerve	3+	—	—	—	—
Trigeminal ganglion	1+	—	±	—	—
Dorsal root ganglion	Neg	—	Neg	—	—
Spinal cord	Neg	2+	±	±	—
Cerebral spinal fluid (cell free)	Neg	Neg	Neg	—	—
Cerebral spinal fluid (with cells)	3+	2+	2+	—	—
Skin	—	—	2+	—	—
Vesicle fluid	—	—	2+	—	—
Thymus	—	—	Neg	—	—

^a Postinoculation day of sampling.^b ± to 4+ denotes the relative amount of DHV cytopathology in Vero cells after one passage of cocultivated tissues.

that viral interstitial pneumonia, encephalitis, and disseminated viral infection occur in experimental DHV-induced simian varicella in the patas monkey. The neuropathogenicity of DHV or other simian varicella isolates has not previously been studied nor has CNS involvement been observed in natural or experimental infections (1, 7). Likewise, simian varicella isolates have not been recovered from the central or peripheral nervous systems of monkeys with simian varicella.

Results in Table I show that all monkeys inoculated with DHV developed severe clinical illness except patas 697. A possible explanation of this result was that the higher cell culture-passaged DHV administered to patas 697 was attenuated since patas 695, which was inoculated SC, IT, and ID in a manner similar to patas 697 be-

came critically ill with pneumonia. Passage of DHV in patas 697 may have selected for a larger proportion of more pathogenic virus which was then recovered from PBL and inoculated to patas 695. It appears likely that DHV inoculation via IT is the best route for producing viral pneumonia. This was indicated by the fact that another patas monkey inoculated SC and ID, but not IT, with low cell culture-passaged DHV did not develop severe illness or pneumonia (data not shown). However, IV or IC inoculation of DHV also produced pneumonia which indicates that the IT route of inoculation was not requisite for inducing pneumonia. Encephalitis with a concomitant pneumonia can be anticipated following IC administration of DHV as indicated in Table I. It is important to note that after IV DHV inoculation of patas 185

virus was recovered from the cerebrum and spinal cord and leptomenineal hemorrhage was present at postmortem. From this result it appears that DHV can reach the CNS without IC inoculation. As a means of differentiating simian varicella virus isolates, it would be significant to know if each isolate would exhibit the same degree of pathogenicity as DHV in the patas monkey. A summary of the comparative microscopic neuropathology of DHV- and VZV-associated CNS involvement is shown in Table II. We conclude from these findings that DHV infection in the CNS of the patas monkey is a simian counterpart of VZV CNS involvement in humans. VZV-associated neuropathology was obtained from several case studies (16, 18, 19, 20) and all of the lesions were not observed in each case.

DHV was isolated from optic nerve of patas 736 and trigeminal ganglion of both patas 736 and 685. Interestingly, no virus was recovered from the dorsal root ganglion of patas 736 or 685.

Cell-associated but not cell-free DHV was isolated from CSF in three monkeys (Table IV). This result indicated that spread of virus in the CNS was probably not facilitated by cell-free virus.

Virological and pathological results of this study and those of others (1, 3, 7, 10) clearly indicate that simian varicella isolates are lymphocytotropic and/or monocytotropic. Virus is readily recovered from spleen, lymph nodes, and Ficoll-Hypaque-banded PBL.

Since DHV was blood borne in PBL the possibility existed that virus recovered from some tissues was present in residual blood even though monkeys were thoroughly exsanguinated. DHV should have been recovered from all tissues if PBL were the source of isolated virus but it was not; for example, a relative 3+ amount of virus was recovered from a very small piece of choroid plexus but none was recovered from a relatively larger piece of liver of patas 685.

Patas 685 developed a widespread vesicular exanthem not observed after DHV infection in the other monkeys. Also, the course of this monkey's clinical illness was

somewhat longer after IC inoculation than patas 733 and 736. Whether or not these differences were related to the fact that a different DHV isolate (NIH, 355) was inoculated in patas 685 remains to be determined.

Previous studies of Felsenfeld *et al.* (8) and Soike *et al.* (10) demonstrated the potential usefulness of the DHV-patas model for therapeutic drug efficacy studies. One important advantage of this animal model as illustrated in this study stems from the fact that encephalitis and pneumonia can predictably be produced and, therefore, the efficacy of appropriate therapeutic intervention for attenuating these complications can be studied. Moreover, important host and exogenous factors can be studied to identify potentially important effects that may affect the development of these severe complications as they occur in human VZV infections.

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