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Identification of Lactate Dehydrogenase-Elevating Virus as the Etiological Agent of Genetically Restricted, Age-Dependent Polioencephalomyelitis of Mice

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The etiological agent of genetically restricted, age-dependent polioencephalomyelitis of mice (the ADPE agent) and several isolates of lactate dehydrogenase-elevating virus (LDV) were compared by biological, physical-chemical, and antigenic criteria. The data indicate that the ADPE agent is a strain of LDV. Like LDV, the ADPE agent induced a selective elevation of plasma enzymes and splenomegaly in mice. The enzyme-elevating activity and the paralytogenic activity of the ADPE agent preparations were shown to belong to the same virus. The ADPE agent demonstrated LDV-like replication kinetics in vivo and in vitro. Moreover, the ADPE agent required primary mouse macrophages for in vitro replication, as does LDV. In turn, the LDV isolates induced a paralytic disease with ADPE-like lesions in the spinal cords of immunosuppressed C58 mice. However, the LDV isolates showed a stronger dependence on strain and age of mouse for the induction of paralysis than did the ADPE agent. The LDV isolates and the ADPE agent exhibited indistinguishable morphologies, buoyant densities, structural protein patterns, and virion ribonucleic acid sedimentation rates. Furthermore, they displayed strong antigenic cross-reactivity, as determined by cross-protection in vivo and by radioimmunoassay.

During studies of the immune response of C58 mice to syngeneic line I_b leukemia, it was found that the injection of inactivated line I_b cells induces a paralytic central nervous system disease in C58 mice that are immunosuppressed by the aging process (20) or by immunosuppressive agents (8). Histopathologically, the disease is characterized by a mononuclear cell infiltration in the gray matter of the spinal cord and brain stem (11, 12). It was subsequently determined (17) that this age-dependent polioencephalomyelitis (ADPE) is actually induced by a lipid-solvent-sensitive, filterable, replicating agent (ADPE agent) which reaches unusually high titers in mice (10^{11} infectious doses per g of tissue). Electron microscopy of tissue extracts from mice with ADPE revealed virus-like particles which are morphologically similar to viruses of the togavirus group. Despite the exceptional ability of the ADPE agent to replicate in mice, its ability to induce paralysis is restricted. Paralysis was induced in immunosuppressed old mice from only 2 of 12 standard inbred strains tested, C58 and AKR (8, 16). Line I_b cells which are maintained in culture (I_bN cells) fail to induce paralysis (J. F. Nawrocki and W. H. Murphy, personal communication), suggesting that the ADPE agent does not replicate in the tumor cells but is copassaged with them in vivo. Since lactate dehydrogenase-elevating virus (LDV) was known to have all of the above characteristics, except paralytogenicity (22, 23, 33), and to be a common serum contaminant of transplantable tumors (4), the ADPE agent was compared by biological, physical-chemical, and antigenic criteria with four isolates of LDV obtained from other laboratories. The data presented indicate that LDV is the etiological agent of ADPE.

MATERIALS AND METHODS

Mice. C58/Wm mice (obtained from W. H. Murphy, The University of Michigan, Ann Arbor, Mich.) and AKR.M/nSn mice (obtained from the Jackson Laboratory, Bar Harbor, Maine) were maintained for Merck & Co. by Buckshire Corp., Perkasie, Pa. Swiss Albino mice were purchased from Lab Supply, Indianapolis, Ind. AKR/J, C3H/HeJ, CBA/J, and SJL/J mice were purchased from the Jackson Laboratory.

Viruses. The four samples of LDV, designated LDV-1 through LDV-4, were isolated by the following investigators: LDV-1 by M. Brinton and P. G. W. Plagemann, LDV-2 by A. L. Notkins, LDV-3 by S. Schlesinger, and LDV-4 by V. Riley. LDV-4 was purchased from the American Type Culture Collection, Rockville, Md. (no. ATCC VR-695), and the other

LDV isolates were kindly provided by the originators. The ADPE agent, which was originally associated with line I_b leukemic cells, was serially passaged in mice in the absence of line I_b cells (17). Stocks of virus were obtained by pooling the plasma of infected mice, 24 h after challenge, and were stored at −70°C.

Enzyme determinations. Plasma was obtained by using heparin or sodium citrate as an anticoagulant and assayed for lactate dehydrogenase, isocitrate dehydrogenase (ICD), glutamatic-oxaloacetic transaminase, and alkaline phosphatase activity, using standard procedures (28, 30). Lactate dehydrogenase and alkaline phosphatase levels are reported in international units (standard milliunits). ICD and glutamic-oxaloacetic transaminase levels are reported in the arbitrary units previously described (18).

Assay for infectivity. Virus titers were estimated by titration of enzyme-elevating activity. The titer was defined as the highest dilution causing enzyme elevation and was reported as median infectious units (50% infectious dose [ID_{50}]) per milliliter. Alternatively, virus titers were estimated by titration of ADPE-inducing activity, using immunosuppressed 7- to 10-month-old C58 mice, as described previously (17). Mice were observed for paralysis for 30 days (8), and titers were reported as ID_{50} per milliliter.

Immunosuppression. Mice were given a single intraperitoneal injection of cyclophosphamide (Cytoxan, Meade-Johnson & Co., Evansville, Ind.) (150 mg/kg) 24 h before infection with virus (17).

Histological methods. Mice were sacrificed with ethyl ether. The brain, brain stem, and spinal cord were fixed in 10% (vol/vol) buffered Formalin. After fixation, representative sections of the midbrain, medulla, thoracic cord, and lumbar cord were processed in the usual manner, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological evaluation.

Test for immunity to ADPE induction. Six-month-old C58 mice were injected with 0.1 ml of either diluted plasma containing 10^5 ID_{50} of virus (as determined by enzyme elevation assay) or diluted normal plasma. Three weeks after immunization, groups of mice were immunosuppressed and then challenged intraperitoneally, 24 h later, with the ADPE agent (10^5 ID_{50}). Mice were observed for paralysis for 30 days.

Electron microscopy. Plasma, collected from Swiss mice 24 h after infection with virus, was diluted 30-fold with serum-free minimal essential medium and clarified by low-speed centrifugation. The virus in the supernatant fluid was pelleted at 100,000 × *g* for 2 h. Pellets were fixed in 2% (vol/vol) glutaraldehyde followed by 1% (wt/vol) osmium tetroxide, stained with uranyl acetate, sectioned, and then stained further with lead acetate (3). Thin sections of infected macrophages were also prepared. Cultured macrophages were infected 8 h after planting, then scraped, and pelleted 14 h after infection. Cell pellets were fixed and stained as described above.

In vitro replication of viruses. Peritoneal macrophage cultures were prepared as described previously (2) from Swiss mice, 4 days after an intraperitoneal injection of 2 ml of 1% (wt/vol) starch solution. Human mononuclear cells were separated from peripheral blood on a Ficoll-Hypaque gradient and seeded in plastic flasks in RPMI medium (34). After 2 h, nonadherent cells were removed by rinsing flasks several times with fresh RPMI. Cell cultures were infected at a multiplicity of approximately 50 ID_{50}/cell (2).

Preparation of radioactively labeled virus. After a 1-h period of virus adsorption, the residual virus inoculum was removed from macrophage cultures and replaced with either minimal essential medium supplemented with 2% fetal calf serum and [5-^{3}H]uridine (20 μCi/ml) or leucine-free minimal essential medium supplemented with [^{3}H]leucine (20 μCi/ml) and fetal calf serum (2%). Culture fluids were harvested 12 to 14 h after infection and clarified by low-speed centrifugation. The fluids were then layered onto a linear 15 to 45% (wt/vol) sucrose gradient and centrifuged at 22,000 rpm for 16 h at 4°C in an SW27 rotor (3). Fractions of 1.2 ml each were analyzed for radioactivity in acid-insoluble material and, in some cases, also for infectivity.

Isolation and analysis of viral RNA by rate-zonal centrifugation. Fractions containing [5-^{3}H]uridine-labeled virus from isopycnic gradients were pooled, precipitated with ethanol at −20°C overnight, washed in ethanol, and resuspended in a 100 mM tris(hydroxymethyl)aminomethane-hydrochloride solution (pH 7.4) containing 2% sodium dodecyl sulfate. Samples of ribonucleic acid (RNA) were layered onto linear 5 to 30% (wt/vol) sucrose density gradients and centrifuged in an SW27 rotor at 14,000 rpm for 16 h at 20°C (3). Fractions of 1.2 ml each were analyzed for radioactivity in acid-insoluble material. Sedimentation rates of RNA were estimated by the method of Martin and Ames (15).

Analysis of intracellular virus-specific RNA. Mouse macrophage cultures in 25-cm^2 flasks were supplemented with actinomycin D (1 μg/ml) 4.5 h after infection and with [5-^{3}H]uridine (20 μCi/ml) (28 Ci/mmol; NEN) 30 min later. After 1.5 h at 37°C, the culture fluid was discarded and the cells were washed with fresh medium. The intracellular nucleic acid was then extracted with 1 ml of a solution containing 100 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.0), 100 mM ethylenediaminetetraacetic acid, 10 mM NaCl, 0.5% sodium dodecyl sulfate, and 300 μg of proteinase K. The samples were incubated at room temperature for 1 to 2 h and then at 37°C for 1 h. After vigorous mixing with a tuberculin syringe, each sample was layered onto a 5 to 30% (wt/vol) sucrose gradient and centrifuged in an SW27 rotor at 14,000 rpm for 16 h at 20°C (3). Fractions of 1.2 ml each were analyzed for acid-insoluble radioactivity.

Isolation and separation of viral proteins. [^{3}H]leucine-labeled virus, pooled from peak fractions from sucrose density gradients, was disrupted in a solution containing 1% (wt/vol) sodium dodecyl sulfate, 1% (vol/vol) mercaptoethanol, and 500 mM urea. The mixture was incubated at 37°C for 1 h. The samples were concentrated by vacuum dialysis, and then the proteins were separated by electrophoresis on 7.5% sodium dodecyl sulfate-polyacrylamide disc gels as described previously (3).

Radioimmunoassay for antiviral antibodies. Fifty microliters of 10-fold serially diluted plasma was added to virus-coated wells of polyvinyl microtiter

plates (3). After 90 min at 37°C, the plates were rinsed three times with phosphate-buffered saline containing 0.1% bovine serum albumin, and then a ^{125}I-labeled immunoglobulin G fraction of rabbit anti-mouse F(ab')$_2$ (kindly supplied by W. Gerhard, Wister Institute; 25,000 cpm in 50 μl of phosphate-buffered saline with 0.1% bovine serum albumin) was added. After an additional 90 min at 37°C, the plates were rinsed again, and the individual wells were analyzed for radioactivity.

RESULTS

LDV-like induction of plasma enzyme elevation and splenomegaly by ADPE agent. The ADPE agent was tested for its ability to induce the elevation of certain enzymes in the plasma of mice, the most distinctive characteristic of LDV (30). Four days after infection, the mice exhibited a pattern of enzyme elevation that was typical of that observed in LDV-infected mice. On the average, infected C58 mice had a 12-fold increase in lactate dehydrogenase, a 10-fold increase in ICD, and a 2-fold increase in glutamic-oxaloacetic transaminase, but no detectable increase in alkaline phosphatase, compared with uninfected C58 mice (Table 1). The ADPE agent induced this pattern of enzyme elevation in C58 mice of all ages and in mice from strains that are resistant to the induction of ADPE, such as AKR.M, C3H, CBA, and SJL.

An experiment was performed to determine whether the two activities of the ADPE agent, paralytogenicity and enzyme elevation, belong to a single infectious agent or to two distinct infectious agents. Eight separate plasma samples from ADPE agent-infected C58 mice were serially diluted 10-fold and injected into normal C58 mice, two mice per dilution. Four days later, plasma samples were taken from these mice. An aliquot was assayed for ICD activity and the remainder of the sample was stored at −70°C. For each of the original plasma samples, a dilution was found which induced one mouse to have an increased ICD level while the other mouse retained a normal ICD level. The stored plasma from these endpoint mice were then tested for paralytogenic activity in immunosuppressed C58 mice. Whereas all of the plasma samples from mice with elevated levels of ICD induced paralysis, none of the samples from mice with normal ICD levels induced paralysis. The probability that two distinct infectious agents would coincide in one of two mice, at the limiting dilution, in eight separate trials is $(1/2)^8$ or 0.0039. Thus, it appears that the two activities belong to a single infectious agent. This hypothesis is further supported by experiments presented below.

The ADPE agent was also tested for its ability to induce splenomegaly in mice, another characteristic of LDV (31). Groups of ten 2-month-old C58 mice were injected with 10^6 ID_{50} of the ADPE agent or with normal mouse plasma. Five days after challenge, the infected mice had a twofold greater average splenic weight (180 mg) than uninfected mice ($P < 0.0001$). Similar results were obtained with other strains of mice.

LDV-like replication of ADPE agent in vivo and in vitro. The following experiment was performed to determine whether mice infected with the ADPE agent display the kinetics of viral replication and enzyme elevation that are peculiar to LDV-infected mice. The amount of ADPE agent in the plasma was determined by titration of both paralytogenic activity (using immunosuppressed C58 mice) and ICD-elevating activity (using normal C58 mice). The two assay methods yielded identical titers. The ADPE agent reached a titer of 10^9 ID_{50}/ml in the plasma of C58 mice within 24 h of infection and then gradually declined (Fig. 1). An increase in plasma enzyme levels was detectable within 2 days of infection and reached its peak within 4 days. This pattern of viral replication and enzyme elevation is typical of that described for LDV (25). Similar results were obtained with other strains of mice.

LDV is also characterized by its ability to

TABLE 1. *Effects of ADPE agent on levels of plasma enzymes*

Treatment of mice[a]	U of enzyme/ml[b]			
	LDH	ICD	GOT	Alkaline phosphatase
Infected with ADPE agent	5,273 ± 1,776	1,516 ± 727	54.8 ± 24.6	17.0 ± 4.2
	(<0.0001)[c]	(0.0002)	(0.0180)	(1.0000)
Uninfected	450 ± 75	150 ± 92	30.5 ± 15.1	17.0 ± 3.3

[a] One group of ten C58 mice was infected intraperitoneally with approximately 10^5 ID_{50} of ADPE agent, whereas a second group remained uninfected.

[b] The level of the indicated enzymes in the plasma was determined 4 days after injection. The average + standard deviation is shown. LDH, Lactate dehydrogenase; GOT, glutamatic-oxaloacetic transaminase.

[c] P value for the difference between the enzyme levels of infected and uninfected mice.

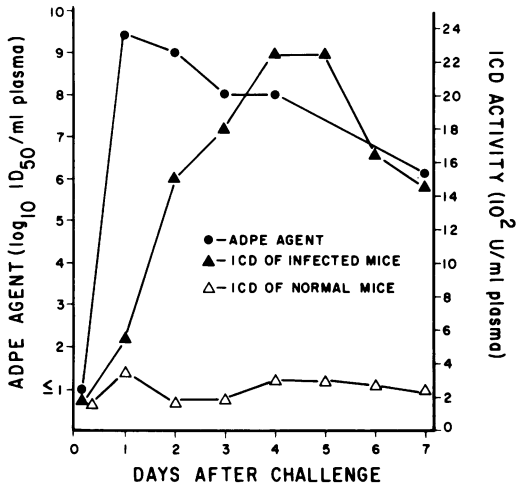

FIG. 1. *Virus titer and enzyme activity in plasma of mice infected with ADPE agent. Groups of six 4-month-old C58 mice each were injected with 10^5 ID_{50} of the ADPE agent and exsanguinated at the indicated times after challenge. The plasma from three mice in each infected group were pooled and titrated for infectivity (●) in mice. The remaining three plasma samples from each infected group were tested individually for ICD level, and the average ICD units per milliliter (▲) was determined. Plasma samples from normal mice (△) were also assayed for ICD activity, one mouse per sampling time.*

replicate in primary mouse macrophages, but not in macrophages from other animals (2, 29). Thus, the ability of the ADPE agent to replicate in mouse peritoneal macrophages and in human blood monocytes was tested. The amount of ADPE agent in the culture fluids was determined at various times after infection by titration of lactate dehydrogenase-elevating activity, using Swiss mice. Figure 2 shows that the ADPE agent replicated well in the mouse cells, but not in the human cells. As observed with LDV (2), the replication of the ADPE agent reached a maximum approximately 16 h after infection and gradually declined thereafter. Moreover, as with LDV, there was no detectable cytopathology in ADPE agent-infected macrophage cultures. Some culture fluids were also titrated for paralytogenic activity, using immunosuppressed C58 mice, and the titers were found to be identical to those determined by titration by lactate dehydrogenase-elevating activity.

Induction of ADPE with isolates of LDV. Four isolates of LDV were tested for the ability to induce paralysis in immunosuppressed 6- and 12-month-old C58 mice. All four isolates induced paralysis characteristic of that induced by the ADPE agent, but the incidence was much lower than that obtained with the ADPE agent (Table 2). Whereas the ADPE agent always induced paralysis in 100% of the immunosuppressed 6- and 12-month-old C58 mice, the LDV isolates generally induced paralysis in only 50 to 60% of the 12-month-old mice and in less than 10% of the 6-month-old mice. Furthermore, the paralysis induced by the LDV isolates had a longer incubation period (Table 2) and was usually less severe than that induced by the ADPE agent

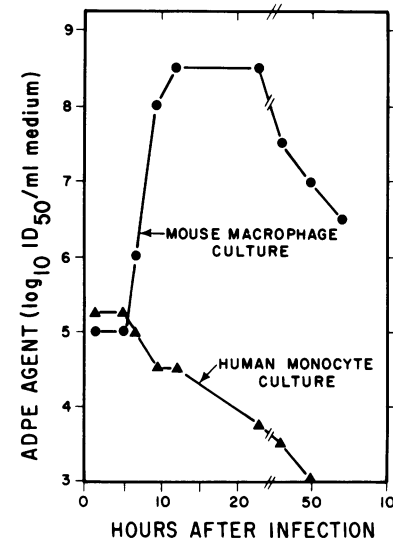

FIG. 2. *Replication of the ADPE agent in mous macrophages. Cultures of mouse peritoneal macrophages (●) or human blood monocytes (▲) were infected with ADPE agent (50 ID_{50}/cell), washed twice with phosphate-buffered saline, fed with RPMI medium containing 2% fetal calf serum, and then incubated at 37°C. One-half-milliliter samples of culture fluid were removed at the times indicated and assayed for infectivity in mice. At each sampling, 0.5 ml of fresh medium was added per culture.*

TABLE 2. *Test for paralytogenicity of LDV isolates in C58 mice*

Virus injected[a]	Incidence of paralysis in:			
	6-mo-old mice		12-mo-old mice	
	Proportion	Mean day + SD[b]	Proportion	Mean day ± SD
LDV-1	0/10		9/11	16.9 ± 5.1
LDV-2	1/10	18	6/10	15.2 ± 5.2
LDV-3	0/10		8/9	17.1 ± 5.2
LDV-4	0/18		6/19	19.0 ± 5.0
ADPE agent	10/10	12.1 ± 2.3	10/10	10.0 ± 0.5

[a] Mice were given cyclophosphamide 1 day before challenge with 10^7 ID_{50} of the indicated virus (as determined by enzyme elevation assay).

[b] SD, Standard deviation.

Approximately 10 to 20% of the LDV-infected mice had a slight impairment of the spreading reflex, which was insufficient to be scored as paralysis.

The LDV isolates failed to induce paralysis in AKR mice, which are less susceptible than C58 mice to the induction of paralysis with the ADPE agent (8), and in mice from several strains (C3H, CBA, and SJL) which are resistant to the induction of paralysis with the ADPE agent (data not shown).

C58 mice that were paralyzed by the various LDV isolates and the ADPE agent developed similar lesions in the spinal cord and, occasionally, in the brain stem. However, the LDV-induced lesions generally seemed less severe and had a slightly lower incidence than the ADPE agent-induced lesions. The lesions (8, 11, 12) were generally confined to the gray matter of the cord but occasionally extended into the surrounding white matter. They consisted essentially of an inflammatory mononuclear cell infiltrate (Fig. 3) that was often more prominent in the ventral horns than in the dorsal horns of the cord. Small mononuclear cell cuffs were occasionally present around blood vessels. Less commonly, there were scattered degenerative changes involving single neurons, and in some sections minute inflammatory cuffs could be seen about rare degenerated neurons.

Electron microscopy of ADPE agent. The size and morphology of the ADPE agent were compared with those of LDV-1. Positively stained thin sections of virus, pelleted from the plasma of infected Swiss mice, and of infected mouse macrophages were examined by transmission electron microscopy. The ADPE agent preparations contained virus particles that were indistinguishable from those in LDV preparations. The ADPE agent preparations had populations of spherical particles, of relatively uniform size, with an average diameter of 55 nm and an electron-dense core of about 30 nm (3). Like LDV (2), the ADPE agent matures by budding from the cytoplasm into the intracytoplasmic vesicles (Fig. 4).

Physical-chemical similarities between ADPE agent and LDV. Recently, LDV has been classified as a togavirus, but it differs sufficiently in its physical-chemical properties from either alpha- or flaviviruses so that it has not been included in either of these groups of togaviruses (3). For instance, the density of LDV is significantly lower in sucrose gradients than those of alpha- or flaviviruses. Hence, the density of the ADPE agent was determined. The

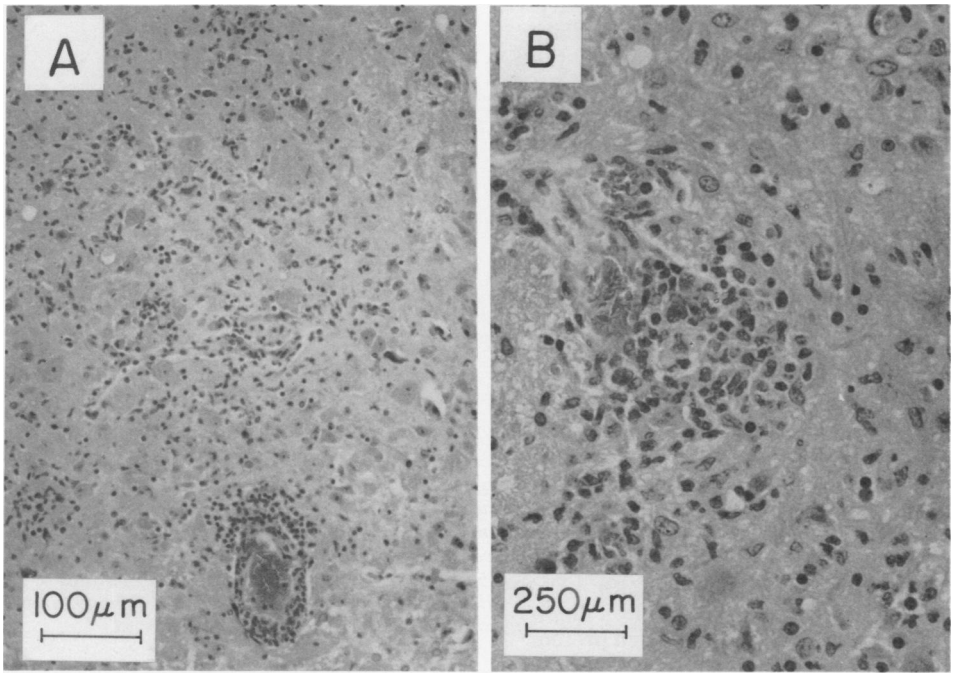

FIG. 3. *Lesions in spinal cords of LDV-infected, immunosuppressed, 12-month-old C58 mice. (A) Inflammatory cells, primarily lymphocytes and microglial cell proliferation, infiltrated the gray matter. Perivascular cuffing was occasionally observed. (B) A few degenerating neurons were surrounded by a relatively dense cuff of mononuclear cells.*

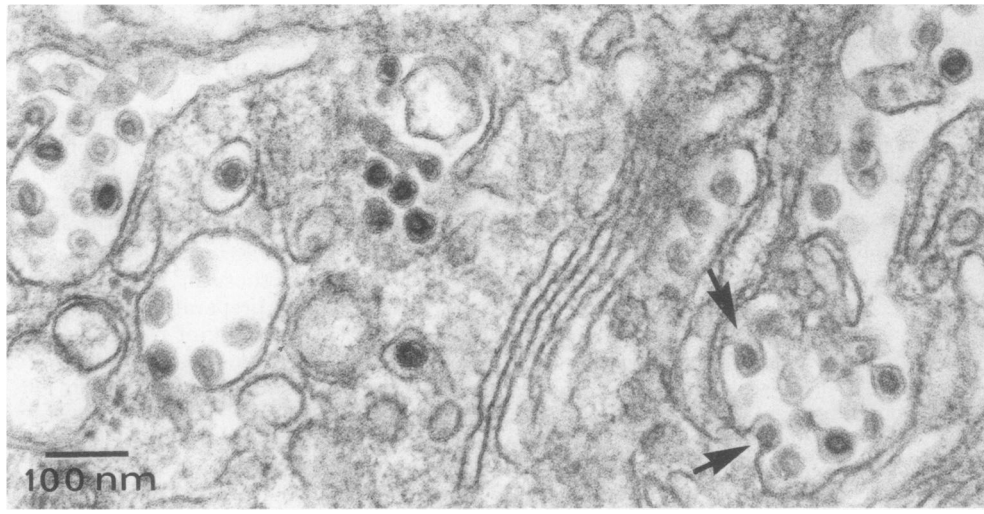

FIG. 4. *Electron micrograph of positively stained thin section of a mouse macrophage 14 h after infection with the ADPE agent; arrows indicate budding virus particles.*

ADPE agent was propagated in primary cultures of mouse peritoneal macrophages in the presence of [5-^{3}H]uridine for 24 h. Culture fluids were then subjected to isopycnic centrifugation in sucrose density gradients. The gradient fractions were analyzed for infectivity (lactate dehydrogenase elevation assay) and radioactivity. Both the radioactivity (Fig. 5) and the infectivity peaked at a density of about 1.13 g/ml, the buoyant density reported for LDV (3). The titer at this density, usually around 10^7 ID_{50}/ml, was the same when determined by both enzyme elevation and paralytogenicity. These results indicate that the ADPE agent is an RNA virus, as is LDV.

The RNA of LDV is a single-stranded molecule with a sedimentation coefficient of about 48S, which is slightly larger than those of alpha- or flaviviruses (3). The RNAs from partially purified LDV-1 and ADPE agent displayed similar sedimentation coefficients (Fig. 6). Also, the sedimentation patterns of virus-specific intracellular RNAs that were produced in mouse macrophage cultures infected with the ADPE agent or with LDV-1 were similar (data now shown).

The glycoprotein(s) of LDV migrates heterogeneously, between 24,000 and 44,000 daltons, on the same gel systems which sharply resolve alpha- and flavivirus glycoproteins (3, 19). LDV also has a non-glycosylated 18,000-dalton membrane protein and a 15,000-dalton capsid protein (3, 19). When the structural proteins of partially purified [^{3}H]leucine-labeled LDV-1 and ADPE agent were separated by electrophoresis on sodium dodecyl sulfate-polyacrylamide disc gels, similar patterns were obtained (data now shown). Indistinguishable patterns are also obtained when the proteins of LDV and the ADPE agent are separated on sodium dodecyl sulfate-polyacrylamide slab gels.

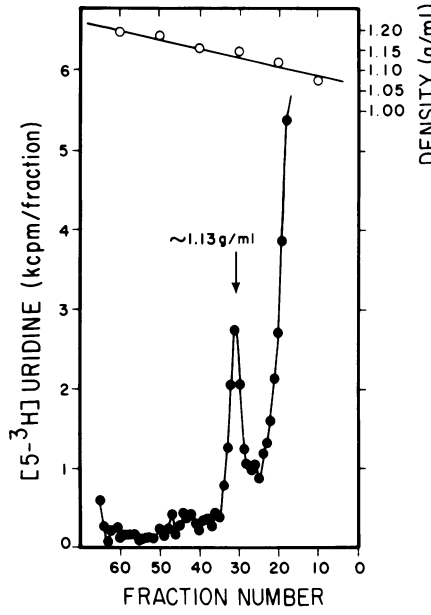

FIG. 5. *Isopycnic sucrose density gradient centrifugation of the ADPE agent. Virus was propagated in cultures of mouse peritoneal macrophages in the presence of [5-^{3}H]uridine. A 5-ml sample of culture fluid was clarified and then centrifuged through a linear 15 to 45% (wt/vol) sucrose gradient. Samples of gradient fractions were analyzed for density and for radioactivity in acid-insoluble material.*

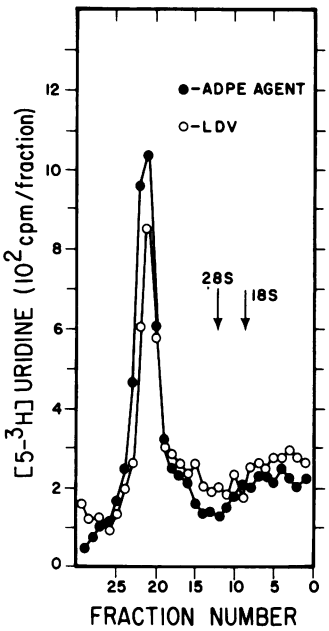

FIG. 6. *Rate-zonal sedimentation analysis of RNA from ADPE agent and LDV. RNA was isolated from partially purified [5-³H]uridine-labeled ADPE agent (●) or LDV (○) and analyzed by zone sedimentation in 5 to 30% (wt/vol) sucrose density gradients. Ribosomal RNAs from BHK cells were used as markers. The positions of the 28S and 18S ribosomal RNAs were estimated from the absorbance profile. Fractions from gradients were analyzed for radioactivity in acid-insoluble material.*

Antigenic cross-reactivity between LDV and the ADPE agent. The injection of immunocompetent C58 mice with the ADPE agent establishes a chronic viremia without the induction of paralysis. These persistently infected mice become resistant to the induction of ADPE by a subsequent immunosuppression-challenge regimen (8). To determine whether persistent infection with LDV would also protect C58 mice against the induction of paralysis with the ADPE agent, groups of ten 6-month-old mice were each injected with one of the LDV isolates and then with cyclophosphamide and the ADPE agent 3 weeks later. Infection with the LDV isolates protected the mice against the induction of ADPE, suggesting that LDV is antigenically cross-reactive with the ADPE agent.

The lack of a suitable serological assay has prevented antigenic classification of LDV. The recent development of a radioimmunoassay for LDV (M. A. Brinton and T. G. Tachovsky, unpublished data) allowed measurement of antigenic cross-reactivity between LDV and the ADPE agent. Plasma from both LDV-1- and ADPE agent-infected mice contained antibody which reacted with both viruses (Table 3).

DISCUSSION

The data presented established that LDV is the etiological agent of the paralytic disease ADPE. LDV and the paralytogenic agent, isolated from C58 mice which had been injected with inactivated line I_b cells, exhibited the same morphological, physical-chemical, and biological properties. Furthermore, the ADPE agent and LDV displayed strong antigenic cross-reactivity. The data also indicate that the enzyme-elevating activity and paralytogenic activity of ADPE agent preparations belong to a single infectious agent. The irregularities that one would expect if two distinct virus populations were present in approximately equal numbers were not observed. Also, each of the four samples of LDV was isolated independently and yet demonstrated homogeneous characteristics.

Although the injection of LDV produces a lifelong viremia in mice (a consistent level of approximately 10^5 ID_{50}/ml of plasma), no clinical symptoms have been previously associated with LDV infections. The infected mice maintain a chronic elevation of certain plasma enzymes (30). This elevation is thought to result from effects of the virus on the reticuloendothelial system, which is involved in the clearance of these enzymes (24). Similarly, LDV infections have a short-lived deleterious effect on the ability of the reticuloendothelial system to clear particulate material (24). LDV infections also

TABLE 3. *Cross-reactivity of LDV and ADPE agent as determined by radioimmunoassay*

Immunoabsorbent	Plasma[a]	Anti-mouse F(ab')₂ (cpm)	Binding ratio[b]
LDV	Anti-LDV[c]	1,387	9.1
	Anti-ADPE[d]	2,631	17.2
	Normal[e]	153	
ADPE agent	Anti-LDV	1,385	9.1
	Anti-ADPE	3,232	21.1
	Normal	153	

[a] The indicated plasmas were diluted and then added to wells that contained the indicated gradient-purified virus. After incubation and washing, ^{125}I-labeled anti-mouse F(ab')₂ was added. The wells were then washed and analyzed for radioactivity.

[b] Counts per minute obtained with antivirus plasma divided by counts per minute obtained with normal plasma.

[c] Pooled plasma from 4.5-month-old Swiss mice, inoculated with LDV at 1.5 months of age.

[d] Pooled plasma from 5-month-old C58 mice, inoculated with ADPE agent at 3 and 4 months of age.

[e] Pooled plasma from 2-month-old Swiss mice.

transiently decrease the number of lymphocytes in the thymus, in the thymus-dependent areas of the spleen, and in the blood (35). LDV-infected mice develop a subclinical glomerulonephritis, which is thought to result from trapping of virus-antibody complexes in the glomeruli (27), as is the case in certain other persistent viral infections (10, 26).

Considering the restricted conditions that are required for the induction of paralysis by LDV, it is not surprising that the paralytogenic activity of LDV was not detected previously. Although the ADPE agent replicates in all of the 13 strains of mice that have been tested, it induces paralysis in only two, AKR and C58 (8, 16). In addition, AKR and C58 mice must first be immunosuppressed by aging or by treatment with immunosuppressive agents, or by both, to render them susceptible to the induction of ADPE (8, 20). The four isolates of LDV, obtained from different laboratories, also induced paralysis in immunosuppressed old C58 mice, but not as efficiently as the ADPE agent. Thus, the ADPE agent appears to be a more paralytogenic strain of LDV. The strain may have been fortuitously selected by prolonged inadvertent passage in C58 mice as a contaminant of the transplanted line I_b leukemic cell suspensions. Line I_b leukemia orignated spontaneously in a 1-year-old C58 mouse in 1929 (13) and has been maintained since then by successive passages in young C58 mice (21). The time at which the leukemic cell line was contaminated with the ADPE agent cannot be determined, since no clinical symptoms are observed in young mice infected with the ADPE agent.

Like other strains of LDV, the ADPE agent appears not to replicate in the tumor cells (28, 29). Thus, when line I_b cells were maintained in vitro (a standard method for freeing tumor lines from LDV), the ability to induce paralysis (J. F. Nawrocki and W. H. Murphy, personal communication) and enzyme elevation (D. Martinez, unpublished data) were lost.

The genetic and immunological factors responsible for the selectivity observed in the induction of paralysis by the ADPE agent (LDV) have been only partially characterized. The resistance of young adult C58 mice to ADPE induction is mediated by a thymus-dependent immune response which can be abrogated by neonatal thymectomy (7), immunosuppressive agents (8), or the aging process (8, 20). Upon challenge with the ADPE agent, immunosuppressed C58 mice maintain higher levels of virus in the tissues than do normal mice (18; J. F. Nawrocki and W. H. Murphy, Fed. Proc. **37:** 1473, 1978). Moreover, the injection of mixtures of immune serum and LDV into susceptible (immunosuppressed) C58 mice prevents the induction of ADPE, suggesting that antibody is involved in the resistance of young C58 mice perhaps by restricting the amount of virus in the central nervous system tissue or by suppressing the inflammatory response, or both (17). On the other hand, the mouse strains that are completely resistant to ADPE induction apparently are not dependent on immunological restriction of LDV for resistance to disease induction, since the resistance of these mice is unaffected by immunosuppression (8, 16). Therefore, the resistant strains must possess an additional genetically determined resistance factor(s) that is non-immunological in nature (16).

Immunosuppressed old SJL mice were found to be resistant to the induction of ADPE (data not shown). LDV-infected SJL mice have been previously reported to display a twofold greater increase in plasma lactate dehydrogenase levels than do other strains of mice tested (5). This trait is inherited as a recessive Mendelian allele (6). The fact that SJL mice are resistant to the induction of ADPE indicates that susceptibility to paralysis induction and the degree of enzyme elevation are controlled by separate loci.

The fact that the two ADPE-susceptible mouse strains have a high incidence of spontaneous leukemia may be more than a mere coincidence and may have implications for certain rare human diseases. It is possible that some of the human viruses, which are usually associated with relatively benign diseases, can give rise to overt diseases in those individuals that are predisposed by genetic factors or an underlying pathological conditions, or both. In this regard, it is noteworthy that several rare human central nervous system diseases, some of which are suspected of having a viral etiology, are associated with malignancy and immunosuppression (1, 9, 14, 36, 37). The similarities and differences between the histopathologies of these diseases and ADPE have been discussed previously (12).

Studies are currently in progress to delineate the mechanism of ADPE induction and the immunological mechanisms involved in resistance to the induction of ADPE.

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