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Chapter 8

Lactate Dehydrogenase-Elevating Virus

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Lactate dehydrogenase-elevating virus (LDV) is a mouse arterivirus, unusual in its extreme host specificity and its persistence in the circulation of the infected host that naturally infects wild mice. Although probably not as frequent in laboratory mouse colonies as it used to be, LDV infection may affect experimental results, primarily through its effects on the host immune responses. On the other hand, because of its unique properties, LDV infections serve as a good animal model for viral persistence, virally induced immunomodulation, and pathogenic infection of neurons in the central nervous system.

II. HISTORY OF ISOLATION

During a search for methods that could be used in the early diagnosis of tumors, Riley and Wroblewski (1960) found that, following the inoculation of mice with Ehrlich carcinoma cells, a 5- to 10-fold increase in lactate dehydrogenase (LDH) levels in the serum occurred before detectable tumor growth. This early rise in LDH levels observed in the tumor-bearing mice was duplicated in normal mice by the injection of serum from the mice with tumors (Riley et al. 1960); serum from these infected mice subsequently raised the levels of LDH in other normal mice. These results suggested the presence of an infectious agent. That this agent is a virus was indicated by its ability to pass through a bacteria-retaining filter and by its susceptibility to inactivation at 70°C. Riley (1968) subsequently found that this virus (LDV) was a contaminant of 26 murine tumors that had been maintained by serial transplantation in mice. However, that LDV is not a necessary component of tumor lines was indicated by the finding that many spontaneous or induced tumors were not contaminated with LDV (Riley 1961, 1962; Crispens 1963; Mundy and Williams 1961; Notkins et al. 1962).

Although LDV has been referred to in the literature by a number of names, including “Riley virus,” “virus enzyme-elevating factor,” and “lactic dehydrogenase virus,” it is now commonly referred to as “lactate dehydrogenase-elevating virus.”
**Fig. 8-1** A positively stained thin section of LDV pelleted from mouse plasma that was collected 24 hours after infection (Brinton-Darnell and Plagemann, 1975).

(Horzinek and Wielink 1975). In sucrose gradients, LDV has an unusually low density (1.13–1.14 gm/ml) (Michaelides and Schlesinger 1973; Horzinek and Wielink 1975; Brinton-Darnell and Plagemann 1975).

The genome of LDV is a 48 S RNA molecule with a molecular weight of 5–6 × 10^6 Da and a length of about 14,000 nucleotides (Godeny et al. 1993). That the RNA is sensitive to ribonuclease (RNase) digestion shows that it is single-stranded. This RNA contains nine open reading frames (ORFs) (Fig. 8-2) (Kuo et al. 1991, 1992; Godeny et al. 1993; Chen et al. 1993). ORF 1 produces two polyproteins, 1a and 1b, that contain conserved protease, RNA polymerase and RNA helicase motifs, and a number of transmembrane domains (Godeny et al. 1993; Faaberg and Plagemann 1996). ORFs 2a through 7 are overlapping and encode structural proteins. Purified viral RNA is infectious when injected into mice intracerebrally, a route that minimizes the chance of RNA digestion by ribonucleases (Notkins and Scheele 1963; Rowson et al. 1968; Darnell and Plagemann 1972; Niwa et al. 1973).

LDV virions likely contain six envelope proteins (Snijder et al. 2003). All but two of these proteins are minor components. The major envelope proteins are GP5 (formerly VP3) and M (formerly VP2). GP5 is a 199 amino acid protein. However, after polyacrylamide gel electrophoresis (PAGE), its molecular weight cannot be estimated with accuracy because it appears as a wide heterogeneous band of 24–44 kDa due to glycosylation (Michaelides and Schlesinger 1973; Brinton-Darnell and Plagemann 1975). GP5 is encoded by ORF 5 and is composed of a short, N-glycosylated ectodomain that contains the major antigenic epitopes and the single neutralization epitope of the virus (Plagemann 2001b), and a longer endodomain (Chen et al. 1993; Faaberg and Plagemann 1995). The other major envelope protein, M, is a nonglycosylated integral membrane protein of 18 kDa, is encoded by ORF 6, and is apparently non-antigenic (Godeny et al. 1993; Chen et al. 1993). GP5 and M form heterodimers linked by disulfide bonds that are essential for viral infectivity (Faaberg, Even, et al. 1995a). The 13–15 kDa nucleocapsid protein, N (formerly VP1), is encoded by ORF 7 (Godeny et al. 1990, 1993; Chen et al. 1993). The molecular ratios of N, M, and GP5 within LDV virions have been estimated to be 3–5:1:1 by Michaelides and Schlesinger (1973) and 2–3:1:1 by Brinton-Darnell and Plagemann (1975).

**Fig. 8-2** LDV genome structure. The nonstructural polyprotein open reading frames (ORFs) 1a and 1b are followed by the E protein gene, three genes encoding minor virion glycoproteins (ORFs 2b, 3, and 4), and the genes encoding the major structural proteins, GP5, M, and N. The cleavage sites in the nonstructural polyprotein are indicated by arrowheads. Black arrowheads indicate sites cleaved by the nsp4 serine protease (S). Cleavage sites for the papainlike proteases (P) and the nsp2 cysteine protease (C) are indicated as white arrows. Four highly conserved domains in ORF 1b are indicated. These are the RNA-dependent RNA polymerase (R), a putative zinc finger (Z), an RNA helicase (H), and a nidovirus-specific domain (X) of unknown function.
D. Intracellular Viral RNA Synthesis and Viral Replication

No continuous cell line has yet been found that is permissive to LDV infection. The synthesis of LDV RNA has been studied in cultures of peritoneal exudate cells in the presence of actinomycin D. Single-stranded 48 S genome RNA and RNase-resistant 27 S replicative form RNA can be detected in infected cells within 3–5 hrs after infection. The time period required for a completed genome to appear extracellularly within a mature virion is about 1.5 hrs at 37°C. Virions begin to be released 5–6 hr after infection (Brinton-Darnell et al. 1975). The synthesis of intracellular viral RNA is completely inhibited by the addition of cycloheximide to the culture medium 0.5–1 hr after infection, indicating that protein synthesis is necessary for the initiation of viral replication. LDV replication involves the formation of a 3' terminal nested set of subgenomic mRNAs in infected cells (Kuo et al. 1991). Negative-stranded subgenomic RNAs have also been detected in infected macrophages (Chen et al. 1994).

The production and accumulation of LDV proteins as well as virus maturation occurs in perinuclear regions. LDV matures by budding from the cytoplasm into intracytoplasmic vesicles (Fig. 8-3) (Brinton-Darnell et al. 1975; Tong et al. 1977; Ritzi et al. 1982; Plagemann et al. 1995). The virions are thought to be released extracellularly by fusion of these vesicles with the plasma membrane.

E. Susceptibility to Temperature and Various Chemical Agents

LDV is most stable in undiluted mouse plasma. However, upon purification or dilution, the virions become more sensitive to inactivation by heating, variation in the salt content of the suspending medium, and treatment with various chemicals. LDV in mouse plasma can be stored indefinitely at −70°C without loss of titer (Notkins and Shochat 1963). However, storage at 4°C results in a loss of infectivity with a decrease in titer of about 3.5 logs in 32 days (Riley 1968). At room temperature, virus-containing plasma or feces retain their infectivity undiminished for about 24 hr. Infectivity is lost more rapidly at higher temperatures. It has been reported that LDV infectivity can be completely inactivated by heating at 58°C for 1 hr (Bailey et al. 1963; Rowson et al. 1966). Virus suspended in

![Fig. 8-3](image-url) Mature LDV particles within intracytoplasmic vesicles and immature particles in the process of budding into these vesicles. Thin sections of cultured mouse peritoneal exudate cells 12 hr after infection with LDV (Brinton-Darnell et al. 1975).
tissue culture fluid is more susceptible to heating at 37°C than is virus in mouse plasma (Evans and Salaman 1965; Riley 1968). LDV infectivity is also strongly reduced by ultrasonic treatment (Cafruny et al. 1995).

LDV is fairly stable between pH 6 and pH 8 but is inactivated at pH 3 (Riley 1968; Crispens 1965a). The virus is inactivated by lipid solvents such as ether, butanol, and chloroform (Notkins and Shochat 1963; Crispens 1965b; Mahy et al. 1966). LDV is extremely sensitive to detergent treatment; a brief incubation with a nonionic detergent, such as 0.01% Nonidet P40 or Triton X-100, is sufficient for the disruption of the virus (Brinton-Darnell and Plagemann 1975). LDV is resistant to digestion with trypsin or papain (Crispens 1965a, 1965b).

IV. VIRUS STRAINS

A. Quasispecies

The etiological agent of a polioencephalomyelitis that develops in some mouse strains (see below) was identified as LDV (Martinez et al. 1980). However, various LDV isolates differ in their ability to induce the disease, as well as by a few oligonucleotides detected by RNA fingerprinting, although their physicochemical characteristics and their structural protein patterns are indistinguishable (Martinez et al. 1980; Contag, Retzel, et al. 1986b; Brinton, Gavin, and Fernandez 1986). Most virus pools obtained from infected mice consist of populations of different virus variants (quasispecies), and most virus pools contain both neuropathogenic and non-neuropathogenic LDV isolates (Chen et al. 1997; Chen, Li, et al. 1998a). Homologous recombination between LDV quasispecies may occur at a high frequency in infected mice (Li et al. 1999). Subsequent sequence analysis of the RNA of biologically cloned neurovirulent (LDV-C, LDV-v) and non-neurovirent (LDV-P, LDV-vx) LDV isolates revealed divergence, especially in the first three ORFs (Kuo et al. 1992; Palmer et al. 1995). Particular differences in the ORF 5 sequence result in differential glycosylation of the GP5 envelope protein of these isolates (Faaberg, Palmer, et al. 1995). Reduced glycosylation of GP5 and sensitivity to antibody neutralization correlate with neuropathogenicity in particular strains of immunosuppressed mice (Chen, Li, et al., 1998b; Chen et al. 2000). Non-neuropathogenic and antibody-resistant virions predominate in chronically infected mice (Monteyne and Coutelier 1994; Chen et al. 1999; Plagemann et al. 2001).

B. Genetic and Antigenic Relationships with Other Viruses

Analyses of the sequence and structure of the LDV genome indicated homology with the genomes of other arteriviruses (de Vries et al. 1992; Meulenber et al. 1993, 1994, 1995; Godeny et al. 1993, 1995; Chen et al. 1993; Conzelmann et al. 1993), but not with those of flaviviruses or togaviruses (Godeny et al. 1989), with which LDV was previously classified. In addition, antigenic cross-reactivity between the primary envelope glycoproteins of LDV and PRRSV was found using antiviral polyclonal and monoclonal antibodies (Plagemann et al. 2002).

V. GROWTH IN VIVO AND IN VITRO

A. Site of Virus Replication In Vivo

That LDV replicates in macrophages has been suggested by a number of different investigations. The replication of LDV is normal in mice subjected to whole-body irradiation (duBuy and Johnson 1966; M. A. Brinton, unpublished observations), which only slightly reduces macrophage number. Using an indirect immunofluorescent technique, two laboratories have demonstrated the presence of cells containing LDV-specific antigen in sections of the spleen and liver (Porter et al. 1969; Rowson and Michaels 1973). Antigen-containing cells were not observed in sections of kidney, lung, thymus, or salivary gland. The maximum number of stained cells (1500–5000 per 6–8 mm²) were observed in spleen sections between 18 and 24 hr after infection. These stained cells contained a nucleus and were located in the red pulp, which suggests that they were monocytes or macrophages. A reduction in the number of nucleated cells in the spleen was observed 36 hr after infection, and only about 200 cells in a 6-8 mm² section showed positive staining. In the liver sections, staining was confined to Kupffer cells (Porter et al. 1969). Electron microscopic examination of the spleens and lymph nodes of infected mice revealed increasing numbers of virus particles 12 hrs after infection that were in close association with the plasmalemma of reticular cells located in the marginal zone of lymphoid nodules in the spleen and medulla of the mesenteric lymph nodes (Snodgrass et al. 1972).

In attempts to locate a target organ, a number of investigators have removed various tissues from infected animals and assessed the titer of virus each contained (Bailey and Monroe 1972; duBuy and Johnson 1966; Plagemann et al. 1963). Such experiments are complicated by the fact the LDV is found in very high titers in the blood. Titers in the spleen, lymph nodes, liver, and thymus are similar to those in the serum, whereas such tissues as kidney, lung, small intestine, pancreas, and brain contain less virus than the serum. Perfusion of the spleen or lymph nodes with saline before titration did not reduce the virus titer significantly (duBuy and Johnson 1966). K. E. K. Rowson (1964, unpublished observations) looked at tissue titers soon after infection and was unable to demonstrate the appearance of virus in any organ prior to its appearance in the serum. These experiments are consistent with the hypothesis that LDV replicates in macrophage-like cells and that such cells are present in many tissues as well.
as in the bloodstream. Although LDV replicates in spleen macrophages, this organ does not represent the major source of circulating virions, since LDV titers in the blood are not affected by splenectomy (Chan et al. 1989).

Neuropathogenic LDV strains have been shown by immunofluorescent analyses, in situ hybridization, and electron microscopy to replicate in anterior horn neurons (Stroop et al. 1985; Brinton, Gavin, and Weibel, et al. 1986; Contag, Chan, et al. 1986). However, viral RNA is detected in these motor neurons only 10 days after infection, while in the same animals, infection of macrophages in lymphoid organs and of cells in the leptomeninges occurs one day after virus inoculation (Anderson et al. 1995).

B. Kinetics of Replication In Vivo

LDV replicates rapidly in mice after infection, reaching an unusually high titer in the serum of $10^{10}$–$10^{11}$ infectious doses 50% per ml (ID$_{50}$/ml) 12–14 hr after infection (Riley 1974; Notkins 1965a). Few other known mammalian viruses replicate as efficiently. The titer subsequently drops to about $10^7$ ID$_{50}$/ml 72–96 hr after infection; thereafter, there is a further gradual decrease until a stable level of $10^5$ ID$_{50}$/ml is reached approximately 2 weeks after infection (Fig. 8-4). The efficiency of LDV replication in mice is demonstrated by the fact that the injection of as little as 10 ID$_{50}$ LDV yields serum titers of $10^6$ ID$_{50}$/ml 24 hr after infection (M. A. Brinton, unpublished observations; Rowson and Mahy 1975). It seems likely that monocytes or macrophages in these cultures are the cells in which LDV replicates, since cultures of adult peritoneal exudate cells, which are rich in macrophages, yield the highest titers of virus. In cultures of peritoneal macrophages, the initial lag period lasts about 5 hours, and a maximum titer of virus of $10^{8.5}$ ID$_{50}$/ml of culture fluid is observed about 16 hours after infection. The production of LDV decreases by about 99% 1–2 days after infection regardless of the age of the culture at the time of infection (Brinton-Darnell et al. 1975; Ritzi et al. 1982). Although a small number of infected cultures may continue to shed virus at a low level, usually the replication of infectious LDV ceases completely and these cultures become resistant to superinfection with LDV. Moreover, infected cells disappear quickly, indicating that LDV replication is cytocidal (Ritzi et al. 1982; Onyekaba, Harty, and Plagemann 1989).

When cultures are infected on successive days after seeding, the virus yield decreases progressively with increasing age of the culture at the time of infection (Evans and Salaman 1965; Brinton-Darnell et al. 1975). When peritoneal macrophages are cultured in the presence of macrophage colony-stimulating factor, a substance present in L cell–conditioned medium (Virolainen and Defendi 1967; Stewart et al. 1975), they retain...
their susceptibility to infection with LDV for several weeks (Lagwinska et al. 1975; Stueckemann et al. 1982).

Even in primary peritoneal exudate cell cultures, only a small proportion of cells seems to be infected. Although 95% of the cells in cultures prepared from starch-stimulated mice are capable of phagocytosis of latex particles, only 6–20% of these cells, when analyzed autoradiographically, showed evidence of a productive LDV infection (Tong et al. 1977). Autoradiographic analysis and electron microscopic examination of thin sections of infected macrophage peritoneal cultures revealed virions in about 3–8% of the cells (Ritzi et al. 1982). However, when macrophages are obtained from 1- to 2-week-old mice, up to 80% of cells are permissive to LDV replication (Onyekaba, Harty, and Plagemann 1989).

Together, these observations suggest that LDV replicates in a subpopulation of macrophages and that virus replication in these cells leads to cell death. Persistence of infection depends on renewal of this cell subpopulation, and this appears to be facilitated by treatment of the cultures with macrophage colony-stimulating factor.

Occasionally, peritoneal macrophage cultures were found to produce very low yields of virus after infection with LDV (M. A. Brinton, unpublished data). This is thought to have been due to an inapparent mouse hepatitis virus (MHV) infection in the mice from which the macrophages were harvested. MHV infections have been shown to alter macrophage ectoenzyme phenotypes and host resistance to a second virus infection (Dempsey et al. 1986).

D. LDV Replication in Cell Lines

Although primary peritoneal exudate cultures support LDV replication, cultures of SV40-transformed macrophages and other murine macrophage cell lines do not (Brinton-Darnell et al. 1975; M. A. Brinton, unpublished observations). Neither primary cultures of rat (Evans 1964) nor human peritoneal macrophages support LDV replication. A number of other cells, such as suckling hamster kidney cells (Tennant and Ward 1962), murine tumor cell lines (Yaffe 1962; Plagemann and Swimm 1966a), HeLa cells (Plagemann and Swimm 1966a), and Rhesus kidney cells (Evans and Salaman 1975), have also been found not to replicate LDV. Only a few LDV-permissive cells have been detected in some clones of cell lines derived from macrophages of 1- to 2-week-old mice (Onyekaba et al. 1989).

One group of investigators has reported that mouse, rat, mink, rabbit, and human cell lines infected with ecotropic, dual-tropic, amphotropic, and xenotropic murine leukemia viruses (MuLV) are permissive to LDV (Inada and Yamazaki 1991; Inada 1993). The ability to infect MuLV-infected cell lines was higher for neurovirulent isolates of LDV than for non-neurovirulent ones, whereas infectivity for macrophages is similar for all LDV strains. This difference correlates with differences in adsorption to the cells, rather than in differences in viral replication, since transfected LDV RNAs from both neurovirulent and non-neurovirulent isolates replicated equally well in MuLV-infected and non-infected cells (Inada and Yamazaki 1991; Inada et al. 1993).

E. Receptor

The identity of the cell receptor(s) for LDV is not known. Pseudotype virions containing LDV RNA and the envelope proteins of mouse hepatitis virus, a coronavirus, could infect cells that are usually resistant to LDV infection (Even and Plagemann 1995), but pseudotype virions containing LDV RNA and the envelope proteins of Sindbis, a togavirus, could not (Lagwinska et al. 1975). These data suggest that the absence of a suitable receptor for LDV on the cell surface represents the major restrictive element to LDV infectivity. This hypothesis was also supported by the efficient infection by LDV of usually resistant cell lines after LDV RNA transfection (Inada and Yamazaki 1991; Inada et al. 1993).

Analysis of the binding of labeled purified LDV virions has shown that one or several trypsin-sensitive molecules, expressed on a macrophage subpopulation and distinct from Fc and C3 receptors, were responsible for LDV attachment to their target cells (Kowalchyk and Plagemann 1985). A decrease in the number of LDV-infected cells detected by immunofluorescence after treatment of cells with anti-Ia antibodies have suggested to some authors a correlation between the expression of Ia antigens by macrophages and their permissiveness for LDV infection (Inada and Mims 1984, 1985a, 1985b, 1987). However, these results could not be confirmed by another group (Buxton et al. 1988), and there is still controversy about the putative role of Ia as the major receptor for LDV. On the other hand, Fc receptors can enhance LDV infectivity for macrophages when the virus has been complexed by antibodies (Cafruny and Plagemann 1982b; Inada and Mims 1985b).

VI. CHARACTERISTICS OF INFECTION

A. Impaired Serum Enzyme Clearance

Increased plasma levels of LDH in mice after LDV infection were responsible for the original discovery of the virus, for its name, and are utilized for the assay of virus infectivity titers. Although there are five naturally occurring LDH isozymes in mouse plasma, only isozyme LDH V has been found in increased amounts in mice infected with LDV (Plagemann et al. 1963; Warnock 1964). As can be seen in Table 8-1, the levels of a number of other serum enzymes are also elevated after LDV infection. Enzyme levels begin to rise by 24 hr after infection, and the maximum increase is observed after 72–96 hr. Although enzyme levels fall somewhat during the next 2 weeks.
after infection, elevated levels persist in infected animals (Fig. 8–4) (Rowson et al. 1963; Notkins 1965a). Observed plasma levels represent a balance between influx and clearance of enzymes. Normally, an increase in the plasma levels of tissue enzymes is the result of cell damage; however, with LDV infection, enzyme levels are permanently raised without evidence of significant tissue damage. Moreover, no increase in LDH levels is observed in LDV-infected cultures of peritoneal exudate cells. The in vivo increase in LDH levels after LDV infection appears to result mainly from a decreased rate of enzyme clearance. The clearance rate has been shown to be significantly reduced in infected animals as compared to uninfected ones (Mahy 1964; Bailey et al. 1964; Notkins and Scheele 1964; Notkins 1965b; Riley et al. 1965; Mahy et al. 1965a, 1965b).

The clearance of many plasma enzymes is due to receptor-mediated endocytosis and involves cells of the reticuloendothelial system, including liver Kupffer cells and other resident macrophages. Increases in the levels of these enzymes likely correspond to cytocidal infection of these cells by LDV (Smit et al. 1990). Interestingly, the SJL/J mouse strain displays a uniquely higher (by about 2-fold) elevation of LDH after LDV infection (Crispens 1971) that is under the control of a recessive Mendelian gene (Crispens 1972). However, the rate of clearance of exogenously injected LDH V was similar in LDV-infected SJL/J and Swiss mice (Brinton and Plagemann 1977), and the reason for the increased LDH levels in LDV-infected SJL mice is not known.

### B. Morphological Changes

LDV infection usually causes no obvious clinical disease. A slight but significant splenomegaly has been reported to occur by 24 hours after infection (Notkins 1965a). A transient fall in the total white blood cell count begins at about 24 hours after LDV infection and lasts for one day (Riley 1968). LDV induces a transitory decrease in thymus weight, starting 24 hours after infection. By 3–4 days after infection, thymus weight, which has decreased by about 40%, then increases again, and by the seventh day exceeds that of the controls (Proffitt and Congdon 1970; Santisteban et al. 1972). Adrenalectomy prior to infection with LDV prevents this involution of the thymus, suggesting that LDV infection causes an increase in the levels of circulating adrenal cortical hormones, which in turn are responsible for cellular loss in the thymic-dependent areas (Santisteban et al. 1972).

Although a significant number of immune complex deposits have been observed in the kidney glomeruli of chronically infected mice, only mild subclinical lesions develop (Porter and Porter 1971) and no symptoms of an immune complex disease are displayed. Significant pathology is induced by LDV infection only in synergy with other pathogenic mechanisms.

### C. Persistence

Although LDV infection results in lifelong viremia, during which the average titer of infectivity in the plasma is $10^5$ ID$_{50}$/ml (Fig. 8–4), infected mice live a normal life span (Notkins 1965a). LDV first replicates in a macrophage subpopulation present in most tissues, and especially in lymph nodes, spleen, and skin (Anderson, Rowland, et al. 1995). At later times after infection, virus is found in the same tissues, as well as in the liver and testis. Persistence of the virus in the blood is thought to be due to the existence in most viral pools of LDV quasispecies that resist neutralization by antiviral antibodies (Monteyne and Coutelier 1994; Chen et al. 1997). As a consequence of the development of an antiviral immune response in the infected host, these resistant LDV quasispecies outcompete antibody-sensitive ones and are predominantly found in chronically infected mice (Plagemann et al. 2001).

### D. Interferons and Cytokines

Although LDV appears to be a poor inducer of type I interferon ($\alpha$-$\beta$-interferon) in vitro (Evans 1970; Notkins 1971a; Yamazaki and Notkins 1973; duBuy et al. 1973), proliferating cells may produce IFN when infected with LDV (Lagwinska et al. 1975). LDV replication is sensitive to the action of interferon in primary mouse embryo cultures (Notkins 1971a; Yamazaki and Notkins 1973), but not in peritoneal exudate cultures (Stueckemann and Plagemann 1978). In vivo, $\alpha$-$\beta$-interferon is produced at high levels about 24 hr after LDV infection (Baron et al. 1964, 1966; duBuy and Johnson 1965; Evans and Riley 1968; duBuy et al. 1973; Koi et al. 1981), and it may be involved in bringing about the decline in plasma viral titers that begins about 24 hr after
infection, as suggested by in vivo experiments that utilized the interferon inducer statolon (Crispens 1970).

Production of type II interferon (γ-interferon) has also been reported in the early hours and days after LDV infection (Plagemann et al. 1995; Markine-Goriaynoff et al. 2002). However, LDV-induced γ-interferon does not control the replication of the virus (Cafruny et al. 1999; Markine-Goriaynoff et al. 2002). LDV permissiveness is reduced in macrophages obtained from mice treated in vivo with γ-interferon, but not in cells incubated in vitro with the same cytokine (Cafruny et al. 1994).

Finally, LDV infection is followed by an early and transient burst of pro-inflammatory cytokines (Fig. 8-5), including interleukins 6 (Markine-Goriaynoff et al. 2001), 12 (Coutelier et al. 1995), 15, and 18 (J. P. Coutelier, unpublished results). The effects, if any, of these cytokines on LDV replication or pathogenesis are not yet known.

E. Cellular Innate Immune Response

The importance of the innate immune system in antiviral defense has been demonstrated during the past few years. Like other viruses, LDV strongly activates cells involved in the innate immune system. Activation of macrophages by LDV leads to the early production of the pro-inflammatory cytokines listed above (Coutelier et al. 1995; Markine-Goriaynoff et al. 2001). Macrophages from LDV-infected mice display an increased potential to produce nitric oxide (Rowland, Butz, et al. 1994). These macrophages also express enhanced levels of Fc and complement receptors (Lussenhop et al. 1982), and have increased phagocytic activity (Stevenson et al. 1980; Meite et al. 2000). However, the ability of these cells to present antigens appears to be impaired (Isakov et al. 1982a, 1982b). Analyses of the number of activated macrophages and of the kinetics of activation indicate that the functions of non-infected cells are stimulated by LDV infection.

LDV infection activates natural killer (NK) cells via the secretion of α/β-interferon (Koi et al. 1981; Leclercq et al. 1987), which in turn leads to an increased production of γ-interferon (Markine-Goriaynoff et al. 2002). A strong enhancement of the cytolytic activity of NK cells is also observed after LDV infection (Koi et al. 1981; Leclercq et al. 1987; Markine-Goriaynoff et al. 2002). However, this activation of innate immune cells fails to control LDV replication in vivo (Markine-Goriaynoff et al. 2002).

F. T Lymphocyte Response

Cytolytic T lymphocytes able to recognize and lyse cells infected with LDV or expressing LDV proteins are activated in the course of an LDV infection (Even et al. 1995). Whether these anti-LDV cytolytic T cells disappear, due to clonal exhaustion, or persist in chronically infected mice is disputed (Even et al. 1995; van den Broek et al. 1997). Stimulation of anti-LDV T helper lymphocytes in the course of infection has been demonstrated by the T-dependence of anti-LDV antibody production (Coutelier et al. 1986) and by the production of anti-hapten antibodies in mice infected with LDV and challenged with an LDV-hapten complex (van den Broek et al. 1997).

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Fig. 8-5 Pro-inflammatory cytokine expression after LDV infection. Expression of IL-6, IL-12, IL-15, and IL-18 messenger RNA was monitored by RT-PCR at different times after LDV infection in the spleen of CBA/Ht mice.
However, these anti-LDV T lymphocyte responses have no effect on the control of virus replication (Onyekaba, Harty, Even, et al. 1989). It has also been reported that LDV infection induces the activation of suppressor T cells able to control the induction of a delayed-type hypersensitivity response to the virus (Inada and Mims 1986a).

G. Antibody Response and Immune Complexes

LDV is antigenic, and xenoimmune antisera, with neutralizing activity, can be obtained after immunization of rabbits with viral particles (Cafruny and Plagemann 1982b). Anti-LDV antibodies can also be elicited after immunization of mice with inactivated LDV or purified nucleocapsid (Coutelier et al. 1986; Cafruny, Chan, et al. 1986; Harty and Plagemann 1988). In infected mice, anti-LDV antibodies are produced as early as 6–10 days after infection (Porter et al. 1969; Cafruny, Chan, et al. 1986). This antiviral antibody production depends on the presence of functional helper T lymphocytes (Coutelier et al. 1986), but only partially on the secretion of γ-interferon (Cafruny et al. 1999; Markine-Goriaynoff et al. 2000). In contrast to antibodies elicited by immunization with the inactivated virus, which are primarily of the IgG1 isotype, most anti-LDV antibodies secreted in infected mice belong to the IgG2a subclass (Coutelier et al. 1986, 1987). While most of these IgG2 antibodies recognize the major envelope glycoprotein, GP5 (VP3), some of them react with the nucleocapsid, N (VP1), but none of them react with the minor non-glycosylated envelope protein M (VP2) (Coutelier et al. 1986; Cafruny, Chan, et al. 1986).

Neutralizing activity for these antiviral antibodies has been demonstrated, but is incomplete (Notkins, Mahar, et al. 1966; Notkins, Mergenhagen, et al. 1966; Rowson et al. 1966; Coutelier and Van Snick 1988). As a result, LDV persists in the circulation of infected mice as infectious immune complexes. These complexes can be further neutralized to some extent by the addition of anti-mouse immunoglobulin antibodies (duBuy and Johnson 1965; Notkins, Mahar, et al. 1966; Notkins, Mergenhagen, et al. 1966; Notkins et al. 1968; Cafruny and Plagemann 1982a; McDonald 1982). Injection of anti-LDV antibodies prevents transplacental transmission of the virus to the fetus (Broen et al. 1992) and polioencephalomyelitis (see below), but not persistence of infection in normal mice. This viral persistence in the presence of antiviral antibodies is due to the existence of LDV quasispecies that escape neutralization (Monteyne and Coutelier 1994; Chen et al. 1997).

The mechanisms of viral neutralization have been analyzed with monoclonal anti-LDV antibodies derived from infected or immunized mice (Harty et al. 1987; Harty and Plagemann 1988; Coutelier and Van Snick 1988). The major neutralization epitope recognized by these antibodies is located on a short hydrophilic segment in the center of the ectodomain of GP5 (VP3) (Li et al. 1998; Plagemann 2001b). Neutralization is impaired by glycans that flank this epitope and that differ between sensitive and resistant quasispecies (Chen et al. 2000; Plagemann 2001b). Neutralization results from the binding of multiple antibody molecules, leading to disruption of the virions (Plagemann et al. 1992). However, prevention of LDV-induced polioencephalomyelitis by antibodies (see below) may rely on other mechanisms, since this prevention is particularly elicited by antibodies of the IgG2a isotype (Markine-Goriaynoff and Coutelier 2002).

H. Modulation of Immune Responses

A depression of cellular immune responses, as indicated by a longer survival of skin or thyroid allografts and a decreased intensity of the graft-versus-host reaction follows infection with LDV (Howard et al. 1969; Isakov et al. 1981). Contact sensitization to 2,4-dinitro-1-fluorobenzene is inhibited (Michaelides and Simms 1977). The proliferative response of T lymphocytes from LDV-infected mice to concanavalin A or anti-CD3 antibody is decreased (Rowland et al. 1994). This depression of T lymphocyte responses could not be attributed to T cell depletion, nor to nitric oxide production by macrophages (Michaelides and Simms 1977b; Rowland, Butz, et al. 1994). However, some of the effects of LDV on T lymphocyte functions, such as depression of secretion of Th2 cytokines, including interleukins 4, 9, and 13, may be triggered by γ-interferon production (Monteyne et al. 1993; Monteyne, Renaud, et al. 1997; Morimoto et al. 1999, 2003). This bias in T helper differentiation may lead to inhibition of antiparasite and allergic immune responses in mice infected with LDV (Morimoto et al. 1999, 2003).

LDV infection suppresses other cellular functions as well. Inhibition of endotoxin-induced inflammation in LDV-infected animals, as measured by the footpad swelling reaction, is mediated by type I interferon (Heremans et al. 1987). The capacity of macrophages from LDV-infected mice to present antigens is decreased (Isakov et al. 1982a, 1982b), although their ability to bind and phagocytose antigens may be enhanced (Michaelides and Simms 1977b; Meite et al. 2000). Stimulation of the formation of germinal centers and an increased level of γ-globulin that does not react with viral antigens have been observed in mice infected with LDV (Notkins, Mahar, et al. 1966; Notkins, Mergenhagen, et al. 1966; Notkins et al. 1968; Cafruny and Plagemann 1982a; McDonald 1982). Injection of anti-LDV antibodies prevents transplacental transmission of the virus to the fetus (Broen et al. 1992) and polioencephalomyelitis (see below), but not persistence of infection in normal mice. This viral persistence in the presence of antiviral antibodies is due to the existence of LDV quasispecies that escape neutralization (Monteyne and Coutelier 1994; Chen et al. 1997).

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humoral defense against the virus. This first humoral response consists of secretion of so-called natural IgS that may have a protective effect before specific antiviral antibodies (including IgM) are produced (Ochsrenbein et al. 1999).

Moreover, LDV infection may modulate antibody responses elicited against nonviral antigens. Most often, this modulation results in a moderate adjuvant effect on these responses, with a shift of their isotypic distribution toward the IgG2a subclass (Notkins, Mergenhagen, et al. 1966; Mergenhagen et al. 1967; Michaelides and Simms 1977a, 1980; Isakov et al. 1982c; Coutelier et al. 1988). However, depending on the mouse strain, on the immunizing antigen, and on the time of infection, LDV may sometimes inhibit concomitant antibody responses (Oldstone et al. 1974; Michaelides and Simms 1980). The repertoire of the antibodies elicited against the immunizing protein may then be changed, resulting in a modification in the proportion of antibodies reacting with either native or cryptic epitopes (Gomez et al. 1997, 2003).

I. Autoimmunity

LDV infection can affect the outcome of concomitant autoimmune diseases, probably via modulation of the immune responses in the infected host. LDV infection in New Zealand (NZ) mice leads to a decrease in the production of antibodies directed against nuclear antigen and red blood cells. In (NZB x NZW)F\textsubscript{1} hybrid mice, which display a high incidence of autoantibodies and a severe immune complex glomerulonephritis with autoimmune hemolytic anemia, LDV infection decreases the amount of antibody synthesized by 4- to 5-fold and significantly reduces the incidence of mortality (Oldstone and Dixon 1972). LDV also suppresses the development of experimental allergic encephalomyelitis induced in SJL/J mice by immunization with spinal cord homogenate (Inada and Mims 1986b). It also prevents the onset of spontaneous diabetes in NOD mice (Takei et al. 1992). The mechanism by which LDV infection suppresses the development of these autoimmune diseases is not known.

LDV infection triggers the spontaneous production of widely different autoantibodies, possibly as a result of polyclonal B lymphocyte activation. These autoantibodies react with various tissues (Cafruny and Hovinen 1988a) and with Golgi apparatus antigens (Weiland et al. 1987; Grossmann et al. 1989), but not with mouse immunoglobulins (Coutelier, Van der Logt, et al. 1990). In CBA/H\textsubscript{t}, but not in BALB/c mice, LDV infection induces the secretion of autoantibodies that preferentially recognize cryptic autoantigens (Gomez et al. 2000). LDV-elicited production of autoantibodies reacting with intermediate filaments may be partly due to antigenic mimicry, since these autoantibodies cross-react with the viral GP5 (VP3) protein (Grossmann and Weiland 1991). Because of its adjuvant effect, LDV infection enhances the production of anti-erythrocyte autoantibodies in mice immunized with rat red blood cells (Verdonck et al. 1994). Finally, through activation of the phagocytic activity of macrophages, LDV strongly increases the pathogenicity of unrelated anti-erythrocyte and anti-platelet autoantibodies, leading to severe anemia and thrombocytopenia, respectively (Meite et al. 2000; Musaji et al. 2004).

J. Polioencephalomyelitis

During studies of the immune response of C58 mice to leukemia cells of the syngeneic Ib line, it was found that polioencephalomyelitis develops after injection of tumor cells either in old C58 mice that become spontaneously immunosuppressed between 6 and 12 months, or in young (3-month-old) experimentally immunosuppressed C58 animals (Murphy et al. 1970, 1983; Duffey et al. 1976; Anderson, Even, et al. 1995). The etiologic agent of this polioencephalomyelitis has been identified as LDV (Martinez et al. 1980; Nawrocki et al. 1980). The disease is characterized by early replication of the virus in cells of the leptomeninges, followed by dissemination into the central nervous system and cytolytic infection of motor neurons of the anterior horn of the spinal cord (Kascak et al. 1983; Stroop et al. 1985; Brinton, Gavin, and Weibel 1986; Anderson et al. 1995b). Lysis of motor neurons leads to progressive paralysis, respiratory failure, and ultimately the death of the infected animals (Schlenker et al. 2001). LDV-induced polioencephalomyelitis may serve as a model for human amyotrophic lateral sclerosis (Murphy et al. 1983; Sillevis-Smitt and de Jong 1989).

Polioencephalomyelitis is not equally induced by all LDV isolates (Murphy et al. 1983). Neuropathogenic and non-neuropathogenic isolates coexist in most LDV pools as quasispecies (Chen et al. 1997; Chen, Li, et al., 1998a; Li et al. 1999; Plagemann et al. 2001) and differ by the number of poly lactosaminoglycan chains attached to the ectodomain of their major envelope glycoprotein, GP5 (VP3) (Faaberg, Palmer, et al. 1995; Chen, Li, et al., 1998b; Chen et al. 2000).

The LDV that was isolated from C58 tumor-bearing mice replicated efficiently in 13 strains of mice tested but induced paralysis only in two, AKR and C58 (Duffey et al. 1976; Martinez 1979). Preliminary studies suggested that control of susceptibility to LDV-induced paralysis might be multigenic and that a gene(s) of the H-2 complex might be involved (Martinez 1979). However, it now appears that the Fv-1 gene, rather than the H-2 haplotype, controls susceptibility to LDV-induced polioencephalomyelitis (Pease et al. 1982; Murphy et al. 1983; Stroop and Brinton 1983). Mouse strains susceptible to the LDV-induced disease are homozygous for the Fv-1\textsuperscript{a} allele, whereas inheritance of a Fv-1\textsuperscript{b} allele confers resistance (Murphy et al. 1983). Moreover, mice with the Fv-1\textsuperscript{a} allele, which differs from the Fv-1\textsuperscript{a} allele by a single nucleotide, are also resistant to LDV-induced polioencephalomyelitis (Monteyne et al. 2000).
The two susceptible mouse strains, C58 and AKR, show a high incidence of spontaneous leukemia, and coinfection with endogenous N-tropic MuLV, which is controlled by the Fv-1 gene, appears to be required for the development of LDV-induced polioencephalomyelitis (Bentley and Morris 1982; Bentley et al. 1982). In situ hybridization analyses suggested that expression of this MuLV in motor neurons is required for their infection by LDV, suggesting that there is a correlation between Fv-1 alleles, MuLV expression, and development of polioencephalomyelitis after LDV infection (Contag and Plagemann 1988, 1989; Anderson et al. 1995a). However, the precise mechanism by which these parameters differentially affect susceptibility to LDV-induced polioencephalomyelitis is not currently known.

Finally, a state of immunosuppression is necessary for the induction of paralysis by LDV in C58 and AKR mice. Also, sensitization with spinal cord tissue may enhance the disease (Stroop and Brinton 1985). Both CD8+ and CD4+ T lymphocytes are required to prevent the development of the polioencephalomyelitis (Bentley and Morris 1982; Bentley et al. 1983; Monteyne, Meite, et al. 1997). Treatment of immnosuppressed mice with IFN-γ protected them against the paralytic disease (Cafruny et al. 1997). Finally, although suppression of the antibody response is not the only prerequisite for the disease (Cafruny, Stranke, et al. 1986), passive transfer of anti-LDV antibodies prevents or delays the progression of this polioencephalomyelitis (Murphy et al. 1983; Harty, Chan, Contag, et al. 1987; Harty and Plagemann 1990). This protective effect is maximal for antibodies of the IgG2a isotype (Markine-Goriaynoff and Coutelier 2002) and correlates with the high sensitivity of neuropathogenic LDV quasispecies to neutralization by antiviral antibodies (Chen et al. 1999).

**K. LDV and Tumors**

LDV has been found in association with more than 50 different transplanted murine tumors (Riley 1968). It is widely accepted that this association is due to chance contamination of a tumor suspension by the blood of an LDV-infected host and that this then leads to the infection of each sequential tumor host with LDV. When mice receive an injection of tumor cells or tumor virus during the first week after LDV infection, an enhancement of the growth and oncogenicity of some tumors is observed. This phenomenon appears to be related to the transient depression in cellular immunity that occurs during the first few days after LDV infection (Howard et al. 1969; Michaelides and Schlesinger 1974).

In contrast, it has been reported that LDV infection delays foreign body (FB) tumorigenesis (Brinton-Darnell and Brand 1977). Mice that received subcutaneous implants of unplasticized vinyl chloride-vinyl acetate copolymer films 2 weeks after LDV infection developed FB tumors at the same 100% incidence as uninfected mice, but at a rate that was slower by 2 months. Since the development of this type of tumor is not affected by immunosuppression (Michelich et al. 1977), it seems unlikely that such a delay in FB tumorigenesis is mediated by the effect that LDV infection has on the cellular immune response. The effect of LDV on FB tumorigenesis may well be mediated through LDV-induced alterations in the monocyte population or in the functions of macrophages (Mahy et al. 1965, 1967; Riley 1974). These cells seem to be intricately involved in the multistage process of FB tumorigenesis (Brand et al. 1975). Finally, LDV-infected mice are protected against the growth of syngeneic Sac tumor cells through the secretion of antibodies reacting with tumor cell surface antigens (Weiland et al. 1990).

**VII. EPIZOOTIOLOGY**

**A. Host Specificity**

LDV is unusual in its extreme host specificity. To date, LDV is known to infect only mice. However, it has been hypothesized that LDV may have infected wild boars that ate infected mice and that this led to the evolution of PRRSV (Plagemann 2003). Viruses with biological properties identical to those of LDV have been isolated from small groups of wild mice in Australia (Pope 1961), Germany (Georgii and Kirschenhofer 1965), the United States (Pope and Rowe 1964; Li et al. 2000), and England (Rowson 1963; Field and Adams 1968). However, nothing is known about the incidence of LDV infection in wild mouse populations. In the laboratory, the virus replicates rapidly in all strains of mice so far tested, producing a persistent infection.

**B. Transmission**

Although LDV replicates rapidly once it enters its host, it is not readily transferred from one mouse to another by natural means. No insect vector for this virus has been identified. Mice infected with LDV have been found to excrete the virus in their feces, urine, milk, and probably saliva (Plagemann et al. 1963; Notkins 1965a; Crispens 1964a, 1964b). However, the oral, ocular, and vaginal mucosal barriers to LDV transmission are relatively effective (Cafruny and Hovinen 1988b), but these barriers are more effective against free viruses than against LDV-infected macrophages (Cafruny and Bradley 1996).

In the laboratory, LDV is rarely transferred between mice, even when they are housed in the same cage. However, it has been reported that, when fighting males were housed in the same cage, virus was apparently transferred via saliva to an open wound (Notkins 1963). In addition, when normal males were housed with infected males whose incisors had been removed, the virus was again transmitted at a high rate, suggesting that
the ingestion of blood and/or tissue of infected animals can lead to infection.

Infected females infrequently transmit the virus to their young. Passage of the virus from the mother to the fetus is maximal between 24 and 72 hours after infection of the mother, and between 12.5 and 15 days of gestation (Haven et al. 1996).

The major mode of transmission of LDV among laboratory mice has most likely been via experimental procedures. Since the virus remains at high titers in the blood throughout the lifetime of an infected mouse, the transfer of serum or tissue from one mouse to another can result in the transfer of virus. The use of the same needle for the injection of several mice can also inadvertently spread an infection.

VIII. DIAGNOSIS

A. LDH Assay

LDH plasma levels increase in all mice infected with LDV. Maximum levels are observed 4 days after infection, but elevated levels persist in chronically infected mice (Fig. 8-4). The most common method for detecting LDV infection is to measure this plasma LDH increase. Small samples of plasma can be obtained from the retro-orbital sinus of mice with heparinized capillary tubes. If plasma samples have a reddish color after centrifugation, they should be discarded, since high levels of cytoplasmic LDH from lysed red blood cells give false positive results.

LDH activity is assayed in a coupled reaction with nicotinamide-adenine dinucleotide (NAD). The activity of LDH can be assessed by measuring either the increase or the decrease in NADH with time:

\[ \text{Lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH} \]

To measure the disappearance of NADH, the reaction mixture containing 2.6 ml 0.5 M phosphate buffer, pH 7.4, 0.2 ml plasma, and 0.1 ml NADH (2.5 mg/ml) is mixed thoroughly in a cuvette, and then 0.1 ml sodium pyruvate (2.5 mg/ml) is added. The decrease in optical density is measured during a 1- to 2-min period at 340 nm. One conventional unit of LDH activity produces a decrease in optical density of 0.001/min/ml plasma. One conventional unit/ml is equal to 0.5 IU. Normal plasma levels of LDH for mice are 400–800 conventional units/ml, whereas levels in LDV-infected mice can range from 1,800–16,000 units/ml. Information on other methods used to measure LDH activity can be found in the review by Rowson and Mahy (1975).

If a sample of mouse plasma is found to contain an elevated level of LDH by the above assay, proof that this increase in LDH was caused by LDV infection can be obtained by intraperitoneal injection of serial dilutions of this plasma into normal mice. Two or more mice are used per dilution, and a sample of their plasma is then obtained on the fourth day after injection and is in turn assayed for LDH activity. A titer of about $10^5$ ID$_{50}$/ml is normal for plasma obtained from a mouse chronically infected with LDV.

B. Detection of LDV RNA, Virions, or Antigens

Contamination by LDV can be detected by amplification of LDV RNA by reverse transcription/polymerase chain reaction (RT/PCR). By using specific primers, for instance corresponding to the LDV ORF 7, it is possible to detect contamination of mice, of cell lines, or of other biological material, including parasites, without false positive results from closely related arteriviruses such as PRRSV or equine arteritis virus (van der Logt et al. 1994). With careful selection of the primers, RT-PCR amplification can be used as a means of detecting LDV quasispecies (Chen and Plagemann 1997; Goto et al. 1998). On the other hand, degenerate primers located in conserved replicase regions can be used to amplify LDV sequences as well as those from other arteriviruses in samples from other host species (Chen and Plagemann 1995).

Several other assays have been developed to detect the presence of either LDV RNA, proteins, or virions in various materials. Viral RNA can be detected on tissue sections by in situ hybridization (Brinton, Gavin, and Weibel 1986). Immunofluorescent studies with anti-LDV antibodies and electron microscopy can be used to detect the presence of LDV antigens and virions in tissue sections (Brinton, Gavin, and Weibel 1986; Stroop et al. 1985). LDV grown in primary mouse peritoneal macrophage cultures in the presence of [5-3H]uridine is detectable by autoradiography in sucrose density gradient fractions (Brinton-Darnell and Plagemann 1975; Tong et al. 1977). After in vivo virus amplification followed by purification of virus from plasma by centrifugation on sucrose gradients, the major LDV structural proteins can be detected by silver nitrate staining after electrophoresis on SDS-polyacrylamide gels (Coutelier et al. 1986; Heremans et al. 1987). Finally, LDV antigens can be quantitated by radioimmunoassay (M. A. Brinton and T. G. Tachovsky, unpublished observations) or by a particle-counting immunoassay based on the agglutination of latex beads coated with anti-LDV monoclonal antibodies (Markine-Goriaynoff et al. 2002).

C. Detection of Anti-LDV Antibody

Measurement of anti-LDV antibody by an enzyme-linked immunosorbent assay (ELISA) has become the method of choice for detecting mice chronically infected with LDV. Using plates coated with purified LDV virions, specific antiviral antibodies can be detected in the serum of infected mice by 1 or 3 weeks post infection (Cafruny and Plagemann 1982a; Coutelier et al. 1986, 1987; Cafruny, Chan, et al. 1986). However, the B lymphocyte
polyclonal activation triggered by an LDV infection leads to the production of antibodies that are not specific for the virus but that bind to ELISA plates not coated with viral antigens (Cafruny, H. et al., 1986; Hu et al., 1992). Specific anti-LDV antibodies can also be assessed by indirect fluorescent antibody staining of infected cells or tissues or by a virus neutralization assay (Cafruny, Chan, et al., 1986), but neither of these methods is used routinely for the detection of infected mice.

IX. CONTROL AND PREVENTION

Because LDV is not very contagious, can escape antiviral immune responses, and infects only mice, it is unlikely that advanced methods of control and prevention of LDV infection, including a vaccine, will be developed. In the case of contamination in animal facilities, LDV-infected mice can be easily detected and euthanized. If a tumor cell line is injected into a mouse with a chronic LDV infection during serial transplantation, LDV will then be transferred with the tumor tissue at each subsequent passage (Riley, 1968). However, since LDV does not replicate in tumor cells in culture, nor in laboratory animal species other than the mouse, it can be eliminated either by passage of the tumor cells in another rodent species or by growing the tumor cells in tissue culture (Plagemann and Swim, 1963, 1966b). The tumor cells must be maintained in culture for several passages in order to eliminate any macrophages or macrophage precursor cells that may be present. Thereafter, the tumor cells can again be maintained in mice. It would be wise to check the LDH levels of recipient mice before injection of tumor cells. Plasma LDH levels should also be assayed 4 days after injection of the cured tumor cells to check that all LDV virions have indeed been eliminated. Likewise, pools of other viruses prepared in mice can be freed of LDV by passage in cultures of continuous cell lines derived from other animal species.

X. CONCLUSION

Although much has been learned about the biology of LDV since its discovery in 1960 by Riley et al., many questions about this interesting virus remain unanswered. The inability of LDV to infect in transformed cell lines and to cause a detectable cytopathic effect in cell cultures in which it does replicate, such as mouse peritoneal exudate cells, represents technical obstacles to the further analysis of this virus. Identification of the cell receptor utilized by LDV would constitute a major advance, since this might lead to the creation of a cell line permissive to LDV infection.

Whereas LDV is a natural and sometimes pathogenic infectious agent of the mouse, it does not currently represent a major threat to animal facilities. The virus is not very contagious and there are a number of easy methods available to detect infected mice. However, additional viruses able to produce chronic infections that are accompanied by no obvious clinical symptoms in most of their hosts may well exist in nature. There is no reason to suppose that such viruses would induce an increase in the host’s serum enzyme levels, the indicator that led to the fortuitous discovery of LDV. The detection of these viruses might well prove difficult. However, such viruses may indeed be responsible for certain effects, that either are currently attributed to known infectious agents or remain unexplained. Further research on LDV infections might thus provide valuable information in fields such as complex infectious diseases, autoimmunity, immunopathology, and oncology. Moreover, further study of the interaction between relatively “silent” viruses such as LDV and their host species should lead to new insights into the mechanisms by which viruses can establish and maintain relatively harmless, persistent infections in their hosts.

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