REVIEW ARTICLE

Lactate Dehydrogenase-elevating Virus

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INTRODUCTION

Lactate dehydrogenase-elevating virus (LDV) was discovered 25 years ago by Dr Vernon Riley† and his colleagues during their work on plasma enzyme levels in tumour-bearing mice (Riley et al., 1960). They found that transplantable tumours of many types caused a five- to ten-fold increase in plasma lactate dehydrogenase (LDH) activity within 3 days of transplantation and before the tumours were clinically obvious. To produce this dramatic increase in plasma enzyme level it was not necessary to transplant cells; cell-free plasma from tumour-bearing mice was equally effective. The raised enzyme level could be serially transmitted from mouse to mouse and proved to be caused by a virus which replicated rapidly in mouse macrophages. Very high titres of viral infectivity (10⁹ ID₅₀/ml) are present in the plasma 24 h after infection, and a stable viraemia at a lower level (10⁴ ID₅₀/ml) is established after 7 to 10 days. This persists for the remainder of the animal's life but does not cause any obvious disease or reduction of life expectancy. The persistent viraemia clearly provides a source of virus for transmission by blood-sucking ectoparasites. It seems likely that this is the method of cross-infection by which the virus is maintained in feral mice, a number of which have been found to be infected in Europe, America and Australia. Transplacental infection often occurs if the mother is infected during pregnancy but is much less likely in chronically infected females (Notkins & Scheele, 1963; Georgii et al., 1964; Crispens, 1967; Motyčka et al., 1976b). The infected male seldom transmits the virus but it is reported that, when an infected male is mated to an uninfected female, there are more females in the litter than expected, although none is infected (Crispens, 1969). The infection can be transmitted by fighting and cannibalism. Laboratory mouse colonies are not often found to be infected but most transplantable mouse tumours and some infective agents maintained by passage in mice are contaminated with the virus. Once contaminated, they can only be freed of the virus by passage in some other species or in tissue culture. Although LDV is not pathogenic in the sense that it does not cause clinical disease in the vast majority of mouse strains, it can cause an alteration in the immune system. One result of this is to alter the growth rate of some experimental tumours and the response to some infectious agents. It is therefore an important virus for the experimenter to be aware of (Riley et al., 1978). With hindsight, it is often possible to see LDV as the cause of experimental results which were at the time inexplicable. Treves and his colleagues (1976) found a tumour growth-enhancing factor in the spleens of mice bearing a transplantable carcinoma. This proved to be LDV (Isakov et al., 1981b). Similar cases are reported by Iorio et al. (1976) and Kamo et al. (1976). Therapeutic results obtained with L-asparaginase in mice were difficult to understand until it was realized that LDV blocks the rapid removal of the enzyme from the plasma. In the uninfected mouse, much larger or repeated doses are required to obtain the same therapeutic effect (Dolowy et al., 1974). Although many of the effects of transplanted tumours on the immune system are due to contamination with LDV, some may not be and it is important to use tumour cells freed of LDV

† Dr Vernon Riley died last year in Seattle, U.S.A. and we would like to dedicate this paper, on a virus which some people have called Riley virus, to his memory.

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(Stevenson et al., 1980; Motyčka et al., 1984a). The role of LDV in mouse pathology is so unpredictable that it should always be considered, although it does not always play a role (Oldham et al., 1980; Cianciolo et al., 1980). The only practical method for detecting the presence of LDV is to observe the dramatically elevated plasma LDH level 3 days after infection. The virus can be detected by some of its other actions such as the development of splenomegaly or interferon induction but these methods lack the specificity of sustained plasma LDH elevation. However, it is interesting that in the 2 years following the first paper on LDV, agents which later proved to be LDV were discovered by observing splenomegaly (Pope, 1961) and interference with vesicular stomatitis virus (Rowe et al., 1962).

There were a number of reviews on LDV in the years before 1975 (see Rowson & Mahy, 1975) but there has been only one since then (Brinton, 1982). The present paper will review the work of the last 10 years on LDV and bring our previous review up to date.

**Virus structure and classification**

Although infectivity is abundant in the plasma of infected mice, LDV has not proved easy to study by electron microscopy. It can be purified (Horzinek et al., 1975; Brinton-Darnell & Plagemann, 1975) and the infectivity correlates with the presence of spherical particles between 62 and 80 nm in diameter, depending on the fixation and staining methods used. The elliptical and rod-shaped particles (de Thé & Notkins, 1965; Prosser & Evans, 1967) and the bizarre shaped particles (Almeida & Mims, 1974) which have been described are probably the result of preparative procedures, as the envelope of LDV is very labile. LDV has a spherical nucleocapsid 35 ± 4 nm in diameter enclosed in an envelope with a smooth surface. Unfixed particles that have been penetrated by phosphotungstic acid reveal an intermediate area between the envelope and the nucleocapsid. The envelope is easily removed with 0-01% Nonidet P40. The intact virus has a density of 1.13 g/ml and the nucleocapsid a density of 1.17 g/ml. The envelope contains two proteins, VP2 of mol. wt. 18000 and a heterogeneous glycoprotein VP3 of mol. wt. 32000. The nucleocapsid is devoid of phosphatidylcholine and contains one protein, VP1 of mol. wt. 15000 (Brinton-Darnell & Plagemann, 1975). The nucleic acid is a single-stranded, infectious RNA of mol. wt. 5 x 10^6. The observation that actinomycin D reduces the yield of infectious virus from mouse embryo cell cultures (Yamazaki & Notkins, 1973) suggested that some host cell DNA-dependent RNA synthesis might be necessary for LDV replication but the action of actinomycin D appears to be a toxic one on the macrophages which have an unusual sensitivity (Brinton & Plagemann, 1979).

On the above data, LDV has been placed in the family Togaviridae, in an unnamed genus with equine arteritis and simian haemorrhagic fever viruses (Brinton, 1980c; Murphy, 1980; Zeegers et al., 1976). It is of the appropriate size and, like many viruses in that family, produces strangely shaped particles on sloughing of the envelope. The RNA is of the correct size and type, very probably a positive strand. The possession of three structural proteins is also a character of Togaviridae but their location in LDV is different from that in viruses belonging to the Alphavirus and Flavivirus genera. The glycoprotein is also different in its apparent heterogeneity. LDV differs from viruses in the Alphavirus and Flavivirus genera in having a significantly lower density, a very much more labile envelope and no projections on the envelope. It resembles other viruses with a lipid envelope in being inactivated by p-nitrophenyl-p-guanidinobenzoate (Bracha et al., 1977).

LDV strains isolated from many different inbred lines of mice appeared to be identical until work on age-dependent polioencephalomyelitis demonstrated that the virus isolated from C58 mice was very much more pathogenic than other strains (Martínez et al., 1980; Nawrocki et al., 1980). Mouse LDV-specific antisera have a very low neutralizing activity and cross-neutralization tests between strains are not easy. Cafruny & Plagemann (1982b) prepared a rabbit anti-LDV serum to their isolate and tested it against six other strains of LDV. Five were poorly neutralized but one was rapidly neutralized at the same rate as the strain used to prepare the antiserum. There thus appear to be at least two serological types. However, a greater degree of cross-reactivity was found by measuring the binding of mouse antibodies induced by the
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various strains to the virus used to prepare the rabbit antiserum. The antigenic difference between the two neutralization types of LDV may not be very stable, as one of the rapidly neutralized strains was derived from a poorly neutralized strain by one passage in Mus caroli. On passage back in M. musculus it produced a marginally lower level of viraemia and plasma LDH activity (K. E. K. Rowson, unpublished data). In an immunodiffusion test using the rabbit antiserum and a Triton X-100-treated LDV preparation, two precipitin bands appeared after 2 weeks at 4 °C but which of the viral proteins was responsible was not determined (Cafruny & Plagemann, 1982b).

Like many viruses in the family Togaviridae, LDV matures by budding through intracytoplasmic membranes. Morphological changes were first seen 3.5 h after infection. Between 1 % and 5 % of the cells contained clusters of membrane-bound and free polyribosomes, with a few double-membrane bound vesicles. The number of such vesicles increased to reach a maximum at 12 to 24 h post-infection, whereas the number of clusters of polyribosomes diminished. Free nucleocapsids were present at 7 h and in greater number by 12 h post-infection. They were interspersed between the double-membrane vesicles and were in various stages of budding into single-membrane vesicles where they accumulated. Infected macrophages were readily identified by the presence of double-membrane vesicles and vesicles containing mature LDV particles. In macrophage cultures 48 h post-infection no infected cells could be found. In sections of splenic tissue from mice infected for 1 day, cells were present with changes similar to those seen in LDV-infected cultures. By 4 days after infection, no signs of infected cells could be detected. The significance of the double-membrane vesicles is uncertain. They have not been reported in any animal cells other than virus-infected mouse macrophages. They appear to play no specific functional role in LDV replication. Although many nucleocapsids are present near the double-membrane vesicles, they mature by budding into single-membrane vesicles located in the same area as the double-membrane vesicles. The formation of double-membrane vesicles may be a response to a viral infection which is characteristic of macrophages rather than to a particular virus, as similar double-membrane vesicles have been observed in macrophages infected by mouse hepatitis virus (Stueckemann et al., 1982b) but not in other cell types. At most, 10 % to 15 % of the cells in a macrophage culture appear to be permissive for LDV replication and these cells are probably destroyed in the process. However, no signs of cell debris have been found but this is not surprising as only a small number of cells are involved and when cell debris is added to a macrophage culture it rapidly disappears (Stueckemann et al., 1982b).

Host range and replication in vitro and in vivo

Viruses with similar characteristics can often be isolated from different host species, but no virus homologous to LDV of mice has so far been found in another species. The host species and cell type specificity of LDV are remarkably narrow. LDV readily infects all strains of wild and laboratory mice, M. musculus, so far tested, but only one other species, M. caroli, has been found to be susceptible to infection (Rowson, 1980). Asian mice in the genus Mus have been divided into three subgenera: Pyromys, Coelomys and Mus (Marshall, 1977). Species in the first two subgenera have not yet been tested but unsuccessful attempts to infect rats, hamsters, rabbits, guinea-pigs, deer mice (Peromyscus maniculatus) and dwarf hamsters (Phodopus sungorus) have been reported (see Rowson, 1980).

As well as having a very limited range of species in which it will replicate, LDV is very specific as to the cell type for replication. It was first shown to replicate in primary mouse embryo cell cultures (Yaffe, 1962) but they had to be freshly explanted. After 7 to 10 days in culture or on subculture they lost their ability to support LDV replication, although there were cells present in which other viruses would replicate. Obviously, a rather special cell was required. Cell cultures from various mouse tissues were tested for their ability to support LDV replication and peritoneal macrophage cultures consistently yielded the highest virus titres in the culture medium (Brinton-Darnell et al., 1975). However, even in macrophage cultures the capacity to support LDV replication declined progressively over a period of a few days as the culture aged. The loss of cells that would support virus replication was most rapid in virus-infected cultures. If the medium was changed every 24 h and titrated for infectivity, there was a rising titre 24 and
48 h after infection but by 72 h the titre was falling. Uninfected cultures also lost their capacity for viral replication but not quite so rapidly, a significant amount of infectivity being produced when a 4-day-old culture was infected. There was thus some evidence that the virus-producing cells were being destroyed in the process but the main cause for the resistance of older cultures to infection was some inherent deterioration in the culture. This deterioration of macrophage cultures can largely be prevented and the cells induced to divide by adding 10% L-cell-conditioned medium, which provides a macrophage growth factor (Virolainen & Defendi, 1967). Cultures maintained with this medium continue to be permissive for LDV replication until they eventually deteriorate after several weeks (Lagwinska et al., 1975; Stueckemann et al., 1982b). For the first 24 h after infection of a macrophage culture there is very rapid virus production, after which it falls away to a very low level but continues as long as the macrophages continue to divide. The phase of rapid virus production is not accompanied by a cytopathic effect and so it was thought that the virus was released without cell destruction. However, it has been shown, using various methods, that at most 15% of the macrophages in a culture are engaged in viral replication (Tong et al., 1977; Stueckemann et al., 1982a). Thus, the destruction of the infected cells could easily occur without producing an obvious cytopathic effect. There was considerable variation between batches of peritoneal macrophages in the percentage of cells producing new virus products but increasing the infecting dose of virus above 50 ID50/cell did not increase the proportion of infected cells (Kowalchyk & Plagemann, 1985). At very low virus doses, 5 to 10 ID50/cell, the presence of LDV antiserum enhanced infectivity (Cafruny & Plagemann, 1982b).

If in fact the virus-producing cells are destroyed in the process, this would explain the sudden end of rapid virus production, which is seen in vivo and in vitro, and the low level of virus production which is maintained in both the mouse and cell cultures during the prolonged chronic phase of the infection. This was previously difficult to explain (Stroop & Baringer, 1982). The termination of rapid virus production in the mouse 24 to 48 h after infection was thought to be due to interferon production (Du Buy et al., 1973; Johnson & Du Buy, 1975) but LDV production in macrophage cultures follows a similar course, although only a small amount of interferon is produced in cell cultures and LDV production is not prevented by high doses of added interferon (Stueckemann et al., 1982a). Another mechanism for the termination of the phase of rapid virus production could be the appearance of temperature-sensitive mutants or defective interfering particles but neither could be detected in persistently infected cultures or chronically infected mice (Stueckemann et al., 1982a). It seems probable that virus production in vivo and in vitro slows down for the same reason: a shortage of permissive cells due to their destruction by the virus.

The mechanism for the restriction of LDV replication to macrophages has been investigated using phenotypically mixed virus particles. In mouse macrophage cultures infected with LDV 7 h before Sindbis virus infection, pseudotype virus particles are formed containing the RNA of Sindbis virus with the envelope of LDV. Such particles are not neutralized by Sindbis virus antiserum but are neutralized by LDV antiserum. Although they have the envelope of LDV, they can infect chicken embryo fibroblasts (Lagwinska et al., 1975). This suggests that the limited host range of LDV is not due to a lack of suitable receptor sites on cells other than macrophages but to a block in replication at a stage after adsorption and penetration. This work with pseudotype virus suggests that there may be cells other than macrophages that are permissive for LDV attachment and replication. It would obviously be valuable to have a cell line in which the virus would multiply and a number of cell lines derived from mouse macrophages have been tested. Although the cells had macrophage properties such as Fc and C3 receptors and the ability to phagocytose and produce lysozyme, none produced significant amounts of virus (Stueckemann et al., 1982b). More promising results were reported, using a hybrid cell line of mouse macrophages and simian virus 40-transformed human fibroblasts. These cells contain all the mouse chromosomes and a single human chromosome 7, but have lost most of the properties associated with macrophages. In spite of this, Schlesinger and her colleagues (1976) reported satisfactory virus replication but they have found it an unreliable system in which to obtain reproducible results (S. Schlesinger, personal communication, 1984).
The replication of LDV in these hybrid cells has been confirmed but only 2% of the cells contained viral RNA; virus titres were quite low in most experiments and never exceeded 10^7 ID50/ml (Stueckemann et al., 1982b).

Although the work on phenotypically mixed virus particles suggests that LDV does not require a specific cell receptor, there is compelling evidence for such a receptor on a small subpopulation of macrophages. Pretreatment of macrophages with trypsin at a concentration of 0.25 mg/ml for 30 min at 37 °C before exposure to LDV prevented their replicating the virus. Exposure to trypsin for 30 min at 1 or 5 h after infection had no significant effect on LDV replication. Macrophages treated with trypsin recover permissiveness for LDV replication about 12 h after removal from the trypsin. This is in contrast to the return of trypsin-sensitive Fc receptors which reappear within 30 min and C3 receptors which reappear in 4 to 6 h. The binding of 125I-labelled LDV to macrophages was competitively inhibited by prior exposure to unlabelled LDV. Labelled virus binding to cells at 4 °C can mostly (78%) be removed by exposure to trypsin, whereas after 30 min at 37 °C only 5% of the radioactivity was removed by trypsin. Although LDV glycoprotein contains mannose and N-acetylglucosamine (Brinton-Darnell & Plagemann, 1975), the virus does not bind to mannose-specific receptors as infection is not blocked by exposure to substrates for these receptors (Kowalchyk & Plagemann, 1985). The Ia antigen is reported to be on a subpopulation of macrophages of similar size to that which is permissive for LDV replication and the Ia antigen has been suggested as the cell receptor for LDV (Inada & Mims, 1984, 1985). However, the evidence is not conclusive and further work is required. As well as removing LDV cell receptors, trypsin destroys viral infectivity without affecting the structural integrity of the virion or altering its density. It does, however, cause the loss of most of the methionine-labelled portion of glycoprotein VP3 (Kowalchyk & Plagemann, 1985).

Silica particles are toxic to the macrophages which take them up (Allison et al., 1966), so injecting them into mice 24 h before infection with LDV might be expected to reduce virus replication. In fact, pretreatment of mice with silica slightly increased the level of plasma virus infectivity 12 and 24 h after infection as compared to control mice not treated with silica (Du Buy, 1975). The explanation may be that the subpopulation of macrophages permissive for LDV replication is not killed but stimulated to divide.

Pathogenesis

Plasma enzyme levels

The increase in activity of certain plasma enzymes in LDV-infected mice is the most striking and easily demonstrated action of the virus. The level of enzyme activity in the plasma is not directly related to the level of viral infectivity (Motýčka et al., 1976a). The enzyme level depends on a balance between the rate of entry and the rate of clearance. It was suggested that LDV might have an effect on both entry and clearance but the evidence is strongly in favour of an effect on clearance only (see Rowson & Mahy, 1975). The clearance rate depends on the activity of the macrophages and on the physicochemical properties of the molecules being cleared. Plasma LDH is a mixture of five isoenzymes with different charges determined by the relative proportions of two polypeptide chains (A and B) in the tetrameric molecule (Appella & Markert, 1961; Jaenicke & Knof, 1968). LDH V (A4) has the highest positive charge and is cleared most rapidly from the plasma. LDH (B4) is negatively charged and has the longest half-life in the plasma. Acetylation of LDH A4 reduces the positive charge and the clearance rate. LDH A4 chemically modified to have the same charge and electrophoretic properties as LDH B4 was cleared at the same rate as LDH B4 (Wachsmuth & Klingmüller, 1978). LDV infection alters the macrophage population and prevents the rapid clearance of LDH A4. It has no action on the clearance of LDH B4. It may be that the subpopulation of macrophages permissive for LDV replication and probably destroyed by the virus are the cells responsible for rapid enzyme clearance of positively charged molecules. The clearance of some plasma enzymes such as aldolase, alkaline phosphatase and acid phosphatase is not affected by LDV infection (Mahy et al., 1964). They may be similar to LDH B4 in not having a fast rate of clearance and may be...
negatively charged molecules. It would be interesting to know whether labelled LDH A4 is only taken up by a small subpopulation of macrophages, the cells in which LDV replicates.

Histological changes

For some years after the discovery of LDV, histological examination of the tissues of infected mice failed to reveal any changes, but an increase in spleen and lymph node mass and a slight transitory fall in thymus weight were reported and confirmed (see Rowson & Mahy, 1975; Stauber et al., 1975; Riley & Spackman, 1974, 1976). The fall in thymus weight may be due to increased activity of the adrenal glands as it can be prevented by adrenalectomy (Santisteban et al., 1972). The increase in spleen weight may not be due to proliferation of splenic lymphocytes but to increased trapping of lymphocytes from the circulation (Isakov et al., 1982a). On very careful examination of the lymphoid tissue, hyperplasia of the germinal centres and a reduction in the concentration of lymphocytes in the thymus-dependent areas was observed (Proffitt et al., 1972; Snodgrass et al., 1972). On the former there is general agreement but Michaelides & Simms (1977b) could not find any evidence of cell depletion in the T-dependent areas.

There is a substantial fall in the total white blood cell count 24 h after infection but it lasts only 24 h before returning to within normal limits. This early and transitory leukopaenia was first reported by Riley (1968) and has been confirmed by Stauber et al. (1975), and by Crispens (1982) who found that the T-lymphocytes were the cells mainly affected. He used SJL/J mice 25 to 30 weeks old and in them he found that the initial fall was followed by a modest increase to above normal level and that there was in the plasma a lymphocyte-proliferating factor which was not interleukin 1 or 2 and was DNase-sensitive.

The presence of circulating virus–antibody complexes very surprisingly seems to produce little tissue reaction (Oldstone & Dixon, 1971; Porter & Porter, 1971). This may be because they do not bind complement except during a brief period round the third week of infection (McDonald, 1982). The only disease-producing pathological changes induced by LDV are in old C58 and AKR mice where there is destruction of lower motor neurones producing age-dependent polioencephalomyelitis (Lawton & Murphy, 1973). The important effects of LDV are alterations which it causes in the functioning of the immune system.

Immunological response

The failure of LDV-infected mice to eliminate virus infectivity from their plasma led to the speculation that antibodies to the virus were not being produced (for review, see Notkins, 1965). However, it was eventually demonstrated that mice do produce neutralizing antibodies but that virus–antibody complexes are formed in which some virus infectivity survives (Rowson et al., 1966; Notkins et al., 1966; Cafruny & Plagemann, 1982a). Free plasma IgG antibodies with specificity for LDV are detectable from about the 6th day after infection (Porter et al., 1969; Cafruny & Plagemann, 1982a) but it is 1 to 3 months after infection before neutralizing antibodies are demonstrable. The neutralizing antibodies present in the plasma of chronically infected mice have a low level of activity. They fail to neutralize at 4 °C and require prolonged incubation at 37 °C (Cafruny & Plagemann, 1982b). They do, however, sensitize the virus to subsequent neutralization by anti-mouse IgG (Notkins et al., 1968). In contrast to mouse antibodies, rabbit antibodies produced in response to injection of concentrated virus with complete Freund’s adjuvant are strongly neutralizing and show single-hit kinetics at 4 °C (Cafruny & Plagemann, 1982b). The reason for the difference between mouse and rabbit antisera is not clear but antibodies produced in mice and rabbits by glutaraldehyde-inactivated virus exhibit the same differences in neutralizing activity (Cafruny et al., 1985). Circulating immune complexes containing immunoglobulin of the IgM class make a brief appearance between the 3rd and 9th days after infection. Complexes containing IgG1 also make a transitory appearance between the 5th and 13th days after infection. The circulating immune complexes which persist indefinitely in the plasma of infected mice contain IgG2 class immunoglobulin and are first present about 15 days post-infection (McDonald et al., 1983).

Notkins and his colleagues (1966) were the first to observe the greatly increased levels of immunoglobulins in the plasma of LDV-infected mice. The increase is mainly in the IgG2a
subclass (Michaelides & Simms 1977a; Coutelier & van Snick, 1985), but all the sixteen IgG fractions separable by isoelectric focusing are increased (Cafruny & Plagemann, 1982a). The increase occurs gradually and might result from a mechanism similar to that which causes the increased plasma enzyme levels but Michaelides & Simms (1977a) found that labelled IgG2a immunoglobulin injected into LDV-infected and uninfected mice was cleared from the plasma at a very similar rate in both. Undoubtedly, some of the increase in immunoglobulin is antiviral antibody (McDonald et al., 1983; Cafruny & Plagemann, 1982a) but much is probably polyclonal and not produced in response to antigen stimulation. That LDV can act as a polyclonal activator has been demonstrated by Michaelides & Simms (1980), who compared the number of antibody-producing cells in the spleens of unimmunized LDV-infected and uninfected mice. They looked for anti-sheep erythrocyte or anti-dinitrophenyl (DNP) plaque-forming cells in the spleens and found a very significant increase in both IgM- and IgG-producing cells 3 days after infection. By 7 days post-infection the difference was less marked and in chronically infected mice there was no increase. Recently Coutelier & van Snick (1985) have produced further evidence for polyclonal B cell activation by LDV. They found that LDV infection selectively stimulated IgG synthesis in mice, and produced a dramatic increase in the proportion of spleen cells giving rise to hybridomas on fusion with SP2/O-Ag14 cells. Most of the hybrids produced IgG2a antibodies and very few (nine of 2549) yielded anti-LDV antibodies. When mice are immunized with glutaraldehyde-inactivated LDV there is no significant increase in total plasma IgG concentration, although the level of antiviral antibody is similar to that in virus-infected mice (P. G. W. Plagemann, personal communication, 1984). In these mice there is no virus replication nor viral antibody complexes and the immune response differs from that in infected mice in that IgM and IgG antibodies appear at the same time; there is no switch from IgM to IgG synthesis after the first week. The polyclonal increase in antibody-producing cells during the acute phase of LDV infection is very transitory and probably not the cause of the sustained increase in circulating IgG1 immunoglobulin. The immunoregulatory potential of LDV infection may be mediated in part at least by the presence of circulating IgG1 complexes, which have been shown to have this capacity (Sinclair, 1978; Voisin, 1980).

Notkins and his colleagues (1966) also noticed an apparent adjuvant action of LDV infection on the humoral immune response of mice to injected human gamma globulin. To obtain an enhanced response, the virus had to be given before the antigen. The optimum interval was 24 h and the effect became rapidly smaller as the interval was increased. In chronically infected mice, i.e. mice infected for more than about 3 weeks, the humoral response is depressed (Oldstone et al., 1974; Riley et al., 1976). The enhanced humoral response to antigen given 24 h after LDV infection has also been demonstrated with two other T-dependent antigens, DNP-bovine gamma globulin and sheep erythrocytes (Michaelides & Simms, 1977a; Isakov et al., 1982a), and with the T-independent antigen DNP-Ficoll (Michaelides & Simms, 1980). The enhancing effect of LDV infection on the humoral antibody level may not be easy to demonstrate; it may only show up in the first few days of the immune response or when the antigenic stimulus is mild. With DNP-bovine gamma globulin as antigen, virus-infected mice produced a greater response than uninfected mice when the antigen was given in saline but there was no difference when the antigen was given with Freund's complete adjuvant (Michaelides & Simms, 1977a).

The enhancing effect of LDV is more easily demonstrated by an increase in the number of antibody-producing cells (Motyčka et al., 1984b). Riley and his colleagues (1976) examined the spleens of mice 48 h after the injection of sheep erythrocytes and found that mice infected with LDV 24 h before challenge with antigen had between three and ten times as many antibody-producing cells per spleen or per 10^7 spleen cells as uninfected mice. The increase is mainly restricted to the subpopulation of antibody-producing cells that form antibodies of the IgG2 isotype (Isakov et al., 1982a). The secondary response is also enhanced but the virus infection must take place before the first dose of antigen. Infection just before the second injection of antigen has no enhancing effect (Isakov et al., 1982a). In mice infected 23 days before injection of antigen, there was a significant depression in the number of antibody-producing cells as compared to uninfected mice (Riley et al., 1976). It would appear that, when an antigen is given shortly after LDV infection, the antigenic stimulus acts synergistically with the viral polyclonal
stimulus to produce the enhanced response observed. The secondary response is also slightly increased as a carry-over from the increased primary response. To affect the secondary response the antigen must be given just before the stage of memory induction (Isakov et al., 1980, 1982a). In chronically infected mice there is no polyclonal activation to increase the number of cells for the antigenic stimulus to work on. The observed reduction in the humoral response seen in chronically infected mice may be due to the destruction of antigen-presenting macrophages by the virus. Many cellular immune responses depend on a T-cell–macrophage cooperation and impaired T cell-mediated responses in LDV-infected mice may be due to an effect of the virus on the macrophages. Antigen-primed T lymphocytes, when challenged \textit{in vitro} by antigen-presenting LDV-infected macrophages, were significantly less responsive than similar cells challenged by uninfected antigen-presenting macrophages (Isakov et al., 1982b, c). The defect in the LDV-infected antigen-presenting macrophages was not due to a failure to take up antigen, as radiolabelled antigen was taken up similarly by macrophages from LDV-infected and uninfected mice (Michaelides & Simms, 1979; Isakov et al., 1982b, c). LDV replicates in macrophages but in only 10% or less of the total, and the infected cells are killed. Thus, when peritoneal macrophages from infected and control mice are compared for a property such as phagocytosis which is a property of all macrophages, it is not surprising that no difference is found but that, when they are tested for antigen presentation, which may be a capacity of a small subpopulation, a difference is found and this may be due to destruction of this subpopulation by the virus. Peritoneal macrophages with Ia antigen may be the antigen-presenting cells and they may be the cells destroyed by LDV. The proportion of Ia-positive macrophages in peritoneal cells from LDV-infected mice is reduced as compared to the proportion in uninfected mice (Hollander et al., 1978; Isakov et al., 1982c).

The splenomegaly induced by LDV infection results from an increase in the number of nucleated cells. This does not appear to be due to replication of the cells in the spleen, as there is no increase in the uptake of \([^{125}\text{I}]\)iododeoxyuridine. It appears to result from an alteration in the circulation of lymphocytes, cells being diverted from lymph nodes to the spleen. If \(^{51}\text{Cr}\)-labelled syngeneic lymphocytes are injected intravenously, the number reaching lymph nodes is reduced and a larger number appear in the spleen in acutely infected animals than in uninfected controls (Mongini & Rosenberg, 1976, 1978; Isakov et al., 1982a). In the acute phase of LDV infection there is also increased trapping of \(^{51}\text{Cr}\)-labelled sheep erythrocytes by the spleen (Isakov et al., 1980, 1982a). The mechanism for the increased splenic activity is not clear but during the acute phase of the infection the subpopulation of macrophages that is permissive for LDV replication produces interferon and this may activate other macrophages to increase Fc and C\(_3\) receptors, thus increasing the phagocytosis of erythrocytes with immunoglobulin or complement on their surfaces (Lussenhop et al., 1982). When normal spleen cells or spleen cells sensitized \textit{in vitro} to sheep erythrocytes are stimulated \textit{in vitro} by exposure to antigen to become antibody-producing, infection with LDV has no enhancing effect on the number of such cells demonstrable in a Mishell–Dutton system or tissue culture of spleen fragments (Isakov & Segal, 1982; Isakov et al., 1982a). Since the virus increases the number of antibody-producing cells \textit{in vivo} but not \textit{in vitro} and has only a limited effect on antibody production, it seems unlikely that it is acting as a simple adjuvant. The polyclonal activation of antibody-producing cells appears to be a primary response to LDV infection but it is transitory. It may account for the increase in antibody-producing cells during the acute phase of infection and the small increase in humoral antibody but the more protracted alterations to the immune response seen in chronically infected mice must result from the altered macrophage population and/or the presence of circulating virus–antibody complexes.

LDV almost certainly plays no part in neoplastic transformation (Isakov et al., 1981a). However, it does have some effect on the progress of a variety of neoplastic conditions. Tumour development may be stimulated or depressed. Michaelides & Schlesinger (1974) made the important observation that it was the timing of the infection that was important. Soon after infection tumour growth was stimulated but in chronically infected mice it was decreased. There are probably several components in the mechanism of action of the virus, and tumours progress over a prolonged period. Under these conditions the details of an experimental procedure can be
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all-important. A minor alteration in timing or dose of tumour cells can alter the result. Unfortunately, in many reports the details of the experimental procedure have not been sufficiently investigated but the general truth of the observations by Michaelides & Schlesinger seems to hold when recent reports of the effect of LDV infection on tumour development are studied (Riley & Spackman, 1976; Brinton-Darnell & Brand, 1977; Isakov et al., 1978, 1981a, b; Henderson et al., 1979; Theiss et al., 1980; McDonald, 1983; Johnson & Shin, 1983). The growth-promoting effect of the acute infection may be ascribed to depression of cell-mediated immunity. Other manifestations of this may be prolongation of survival of allografts (Howard et al., 1969; Isakov et al., 1981b), inhibition of graft versus host response (Howard et al., 1969), decreased autoimmunity response in NZB mice (Oldstone & Dixon, 1972), depression of contact sensitivity (Bendinelli & Asherson, 1971; Michaelides & Simms, 1977b; Vincent et al., 1977), decreased resistance to murine malaria (Henderson et al., 1978) and to infection with Listeria monocytogenes (Bonventre et al., 1980; Isakov & Segal, 1983). It has recently been suggested by McDonald (1983) that the circulating virus-antibody complexes may be the mechanism depressing cell-mediated immunity and that the transitory appearance of complexes containing IgG1 in the early stages of the infection may be particularly important.

Age-dependent polioencephalomyelitis

LDV was considered to be non-pathogenic until its association with age-dependent polioencephalomyelitis was reported (Nawrocki et al., 1980; Martinez et al., 1980). This motor neurone disease of mice was first seen during experiments with the Ib transplantable leukaemic cell line in C58 strain mice (Murphy et al., 1970). Animals ranging in age between 1 and 13 months were given an immunizing dose of 107 formalin (1:5000)-inactivated Ib cells, followed 15 days later by a challenge dose of 103 viable cells. The majority of mice aged 6 months or younger had responded to the immunizing dose and survived the challenge. The older mice showed a progressive inability to develop a presumably cell-mediated resistance to the growth of the transplanted Ib cells. Many of the mice over 6 months of age that received an immunizing dose of cells developed a paralytic disease after 7 to 14 days. The first signs of this disease were impairment of the spreading reflex of the hind limbs accompanied by impairment of the grasp reflex and soon followed by a flaccid paralysis of the hind legs. In some mice the disease was mild and the signs unilateral, and recovery occurred in 1 or 2 weeks. More commonly, both hind legs were paralysed, the disease progressed to involve the fore legs and death followed, presumably from respiratory failure. Ataxia, tremors or seizures were not seen. Animals with paralysis confined to the hind legs could be kept alive if they were provided with accessible food and water. On gross examination, the brain and spinal cord did not show abnormalities. Histologically, there was destruction of neurones sharply limited to the grey matter, with no evidence of meningitis or demyelination. Ganglia, nerve roots and the proximal parts of the peripheral nerves were free of histological changes. There was some perivascular infiltration with chronic inflammatory cells. These changes could not be seen before the 6th day after injection, were most severe by the 8th day and then did not become more marked (Lawton & Murphy, 1973; Homburger et al., 1973).

The only strains of mice susceptible to age-dependent polioencephalomyelitis are C58 and AKR. They only become susceptible when over the age of 6 months and their susceptibility increases thereafter with advancing age. Their susceptibility can be increased or younger mice made susceptible by exposure to an immunosuppressive dose of whole body X-irradiation or drugs. Cyclophosphamide or 600 R given 24 h before challenge were the most effective but prednisolone or methotrexate were also effective (Duffey et al., 1976b). The disease proved to be transmissible by the injection of serum from affected animals but only mice over 6 months of age were susceptible (Sanger et al., 1973). It was thought that the causal mechanism was a 'serum-mediated immune response', an autoimmune response of C58 mice to an antigen common to central nervous system tissue and lymphoid cells (Morris & Murphy, 1978; Murphy, 1979). It was therefore called immune polioencephalomyelitis. However, young C58 mice could be made susceptible by immunosuppression (Duffey et al., 1976b). This argued against an autoimmune hypothesis and in favour of an infectious agent. Early work had not indicated that a
viral infection was a likely cause (Murphy, 1979) but the use of old immunosuppressed C58 mice as a test system provided a sensitive and reliable assay for the causal agent. This made it possible to titrate the agent and it was found that tissue extracts from affected mice had a very high virus titre. The agent could be serially passaged, was shown to be filterable and was sensitive to lipid-solvents. Virus-like particles 40 nm in diameter were present in tissue extracts. Young mice resistant to the disease produced ‘antibodies’ which were protective in old sensitive mice (Martinez et al., 1979; Murphy et al., 1980). The causal agent was established as a virus which soon proved to be LDV (Martinez et al., 1980; Nawrocki et al., 1980; Bentley et al., 1982).

Although LDV is the causal agent, the disease only appears in C58 and AKR mice which are old and/or immunosuppressed. Genetic studies of the common inbred mouse strains suggested that susceptibility was not linked to the major histocompatibility complex but correlated with the Fv-1a allele. The presence of multiple copies of N-tropic C-type retrovirus also appeared to be a factor in susceptibility to the disease (Pease & Murphy, 1980; Pease et al., 1982). The Ib tumour-associated surface antigen does not appear to be involved (Bentley et al., 1982). The thymus plays some role in the resistance of young mice to LDV-induced paralysis, as neonatal thymectomy increases their susceptibility to the disease (Duffey et al., 1976a). Immunosuppressed old C58 mice can be protected from LDV-induced paralysis by the injection of 107 spleen cells from 4- to 5-week-old uninfected C58 mice. The important cells appear to be a subset of T lymphocytes of antigenic type Lyt-1,2; probably suppressor cells (Bentley & Morris, 1982; Bentley et al., 1983; Murphy et al., 1983). The susceptibility of C58 mice and the effect of advancing age or cyclophosphamide do not seem to be due to a failure in humoral antibody production (Cafruny et al., 1986) but to be related to a failure in suppressor T cell function. Cyclophosphamide appears to have a preferential effect on these cells (Turk & Parker, 1982). It may be an action similar to advancing age: the impairment of suppressor T cell function. However, the mechanism by which depressed suppressor T cell function could render mice susceptible to paralysis triggered by an acute LDV infection is not clear. C58 mice, especially when treated with cyclophosphamide, have a slightly higher virus titre in their plasma than other strains of mice and they have a rather higher proportion of macrophages permissive for LDV replication (Cafruny et al., 1986). There is no evidence of LDV replication in the lumbar cord of C57BL/J mice, the virus titre never rising above 103 ID50 per g of tissue. This level of infectivity is probably due to plasma virus contamination. In contrast to this, in C58 mice the virus titre in the lumbar cord rises rapidly from day 3 after infection to reach a peak level of 106 ID50 per g of tissue by the 10th day post-infection (Kascak et al. 1983). The high virus titre in the lumbar cord correlated with clinical disease. Animals with only hind leg paralysis had a high virus titre only in the lumbar cord, whereas mice with hind and fore leg paralysis had high virus titres in both lumbar and cervical sections of the cord (Kascak et al., 1983). Electron microscopy revealed LDV-like particles within neurones (Brinton, 1981). Paralysis is associated with an inflammatory reaction and neuronal destruction. As LDV is known to replicate in macrophages, it could be replicating in the cord as a result of the presence of the inflammatory cells. However, Kascak and his colleagues (1983) treated mice in the early stages of LDV infection with immunosuppressive drugs and so prevented the inflammatory reaction in the cord developing. This did not prevent the onset of paralysis or the high titre of LDV in the lumbar cord of paralysed mice but further work is required as the interpretation of LDV levels in tissues is difficult because of plasma contamination and the uncertainties in assessing the effect of tissue perfusion (Cafruny et al., 1986). By immunofluorescence, viral antigen can be demonstrated in the anterior horn neurones but LDV virions have not been seen (Stroop et al., 1985). It thus appears that LDV may be replicating in and destroying the neurones. An acute infection, with the consequent high level of infective virus in the plasma, seems to be a necessary factor in producing paralysis (Brinton, 1980a, b). LDV infection of young C58 mice before they are susceptible to polioencephalomyelitis gives them immunity to the disease later in life. This is probably not due to antibody production but to the chronic infection which prevents a high level of viral infectivity in the plasma being attained. In the chronically infected mouse there are too few permissive cells to provide the high virus titre necessary to trigger the mechanism, whatever it may be that leads to central nervous system damage. Susceptibility may not be an all-or-
nothing state; it has recently been reported that immunosuppressed LDV-infected young C58 or AKR mice develop histological changes in the cord without paralysis. Changes were also detected in C57 mice and the changes seemed to be progressive. The relationship of these changes to the more acute and severe disease is unclear (Stroop & Brinton, 1983; Stroop et al., 1985), as is the role of LDV in epidermoid cysts of the spinal leptomeninges (Stroop, 1984).

CONCLUSIONS

The physical nature of LDV is now fairly clear, although it awaits allocation to a named genus in the family Togaviridae. The evidence that it replicates in a special group of macrophages and destroys them in the process is very strong. The reason for the permissiveness of this small subpopulation of macrophages is probably related to the presence of trypsin-sensitive virus receptors on the cells. These may well be Ia antigen but there is some evidence from work with phenotypically mixed virus particles which suggests that the failure of most mouse cells to become infected by LDV is not the lack of cell receptors but some block after virus adsorption and penetration.

The acute phase of LDV infection involves the destruction of virtually all the cells permissive for LDV replication and an enormous release of new virus within a period of 12 to 18 h. The rapid killing of the virus-infected cells explains the sudden termination of the phase of rapid virus production in both mice and cell cultures. It is a reasonable expectation that the permissive cells which have been destroyed will be replaced and will then in turn be destroyed, with further virus production. In this way the stable viraemia which persists indefinitely at a lower level than that found soon after infection can be explained. It is a classic self-regulating feedback system. The permissive macrophages which replicate LDV presumably have some other function and, as their number is very much reduced in chronically infected mice, it may well be that some of the processes that are altered in the LDV-infected mouse are carried out by these cells. The most obvious is the rapid removal of certain positively charged enzymes from the plasma. Another may be the presentation of antigen, the failure of which could account for the depressed humoral response in chronically infected mice. In the first day after infection, during rapid virus production there is a transitory production of interferon which may account for some of the changes associated with the acute infection. There is also a polyclonal stimulation of antibody-producing cells but the mechanism for this is not clear. However, given this increase in antibody-producing cells, it is not surprising that an antigen given at this time should elicit an increased antibody response. The polyclonal stimulation of antibody-producing cells is transitory and so in the chronic phase of LDV infection there is no increased humoral response. There is, in fact, a decreased response, as mentioned above. During the acute phase of infection there is a transitory depression of cell-mediated immunity and phagocytosis. How the virus produces this depression is not clear but the reduced cell-mediated immunity can account for many of the biological alterations which have been reported in LDV-infected mice. In chronically infected mice the cell-mediated immunity has returned to normal.

The viraemia and reduced numbers of permissive macrophages are stable changes which persist. With the appearance of antibodies another stable change occurs: the formation of virus–antibody complexes. These may be involved in stimulating polyclonal antibody production, which may in turn be involved in the depressed growth of tumours in chronically infected mice.

The association of LDV with age-dependent polioencephalomyelitis is remarkable, as this virus has not previously been associated with clinical disease of any kind. Only a few strains of mice are susceptible and their susceptibility depends on a loss of a subpopulation of Thy-1,2+ T cells. This occurs with advancing age from 6 to 12 months or on treatment with cyclophosphamide. When these special T cells are sufficiently reduced in number, an acute LDV infection with high virus titre in the plasma results in clinical paralysis. The virus titre in the cord rises over a period of a few days but whether the neurones are productively infected has not yet been reported. Early work suggested the involvement of an autoimmune process and/or an endogenous retrovirus but the evidence is not compelling.

We are indebted to Peter Plagemann, who generously provided data and manuscripts in advance of publication, and to Hazel West for accurate preparation of the typescript.


