VIROLOGY 242, 239–245 (1998)
ARTICLE NO. VY979014

The Neutralization Epitope of Lactate Dehydrogenase-Elevating Virus Is Located on the Short Ectodomain of the Primary Envelope Glycoprotein

Kehan Li, Zongyu Chen, and Peter Plagemann[1]

Department of Microbiology, Medical School, University of Minnesota, Box 196 FUMC, Minneapolis, Minnesota 55455

Received May 22, 1997; returned to author for revision August 6, 1997; accepted December 24, 1997

We have measured by indirect ELISA the binding of neutralizing and non-neutralizing anti-lactate dehydrogenase-elevating virus (LDV) polyclonal and monoclonal antibodies to synthetic peptides representing unmodified hydrophilic segments of LDV proteins. Using this method a single neutralization epitope has been shown to be located in the very short (about 30 amino acid long) ectodomain of the primary envelope glycoprotein, VP-3P, encoded by ORF 5. Although the neutralization epitopes of neuropathogenic and non-neuropathogenic LDVs differ slightly in amino acid sequences, the neutralizing antibodies bind strongly to the epitopes of both groups of viruses. However, the neutralization epitopes of neuropathogenic and non-neuropathogenic LDVs are associated with different numbers of polylactosaminoglycan chains (1 and 3, respectively) which may affect the binding of neutralizing antibodies to the virions of these LDVs. The ELISA using synthetic peptides containing the neutralization epitope provides a novel, rapid, sensitive, and inexpensive method for quantitating LDV neutralizing antibodies in infected mice.

Lactate dehydrogenase-elevating virus (LDV) is a member of the *Arteriviridae* (Cavanagh, 1997). LDV normally establishes a lifelong persistent infection in its only identified host, the mouse (Plagemann, 1996), which is maintained by continuous rounds of cytocidal replication in a subpopulation of macrophages in spite of humoral and cellular anti-LDV immune responses (Plagemann, 1996; Plagemann *et al.,* 1995). The infection is asymptomatic, except for a 5-10 fold elevation in plasma lactate dehydrogenase (LDH) activity which is invariably associated with an LDV infection (Plagemann and Moennig, 1992; Plagemann, 1996). In addition to infecting macrophages, some isolates of LDV can cytocidally infect anterior horn neurons in certain mouse strains, such as AKR and C58, and thus cause paralytic disease (age-dependent poliomyelitis [ADPM]; Martinez, *et al.,* 1980; Murphy *et al.,* 1983; 1987; Anderson *et al.,* 1995b). However, infection of the central nervous system (CNS) by these neuropathogenic LDVs is blocked by anti-LDV immune responses (Harty and Plagemann, 1990; Anderson *et al.,* 1995a).

Recent experiments have shown that various original isolates of LDV, such as LDV-PLA (Brinton-Darnell and Plagemann, 1975) or LDV-RIL (Riley *et al.,* 1960), consist of at least two different non-neuropathogenic LDV quasispecies, LDV-P and LDV-vx, whereas populations of the original neuropathogenic LDV-C-BR (Martinez *et al.,* 1980) and LDV-VIR (Anderson *et al.,* 1995a) were found to contain neuropathogenic LDV quasispecies LDV-C and LDV-v, respectively, in addition to non-neuropathogenic LDV-vx and/or LDV-P (Chen *et al.,* 1997; Chen and Plagemann, 1997). The different quasispecies have been identified and distinguished by sequence analyses of ORFs 2 and 5, and differential reverse-transcription (RT)- polymerase chain reaction (PCR) assays that are based on sequence differences in ORF 2 and ORF 5 (Chen *et al.,* 1997; Chen and Plagemann, 1997). By repeated end point dilutions in mice we have biologically cloned the two neuropathogenic LDVs, LDV-C and LDV-v and the two non-neuropathogenic LDVs, LDV-P and LDV-vx (Chen *et al.,* 1997, and unpublished data). These cloned LDV quasispecies have allowed us to further explore phenotypic differences between the non-neuropathogenic and neuropathogenic LDVs and the molecular mechanisms responsible for these differences. The neuropathogenic LDVs have been found to possess an impaired ability to establish long-term persistent infections in either ADPM-susceptible or non-susceptible mice (Chen *et al.,* 1997). For neuropathogenic LDVs, both the blockage of the infection of the CNS by anti-LDV immune responses and their impaired ability to establish a persistent infection could be due to an increased susceptibility of these LDVs to humoral immune responses of the host.

In the course of testing this hypothesis we have determined that the neutralization epitope of LDV is located on the very short ectodomain of the primary envelope glycoprotein, VP-3P (encoded by ORF 5), using a novel synthetic peptide ELISA. We also demonstrate that the neutralization epitopes of the non-neuropathogenic and

[1] To whom correspondence and reprint requests should be addressed.

0042-6822/98 $25.00

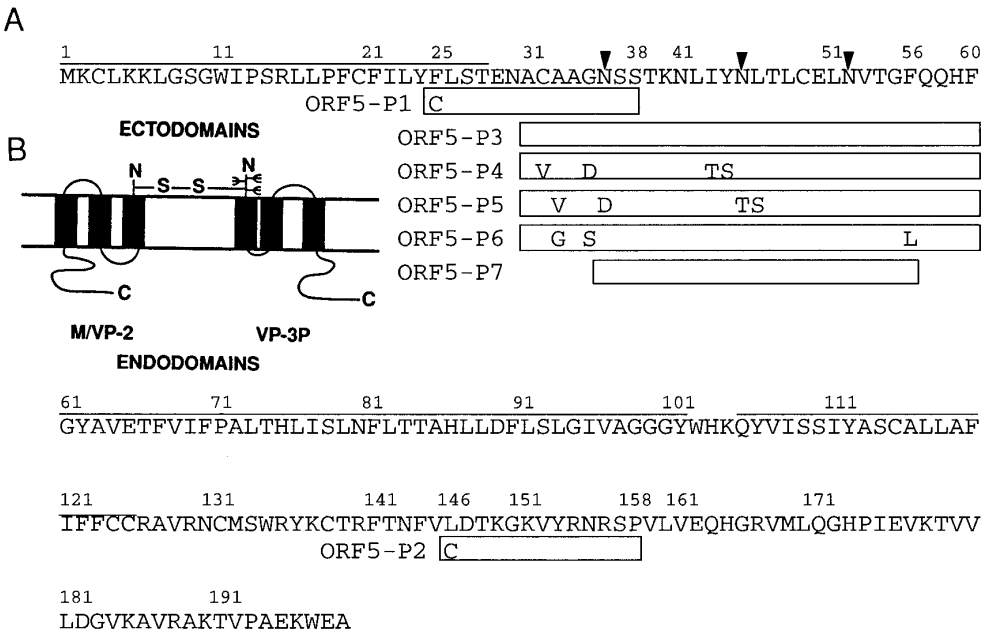

FIG. 1. Amino acid sequences of the ORF 5 protein (VP-3P) of LDV-P and of ORF 5 specific peptides P1–P7 (A) and a model for the topography of VP-3P and M/VP-2 of LDV-P in the virion envelope (B). (A) The signal peptide and the putative transmembrane segments are overlined and the potential N-glycosylation sites in the ectodomain are indicated by ▼ (Palmer *et al.,* 1995). The synthetic peptides to the ORF 5 protein are shown in boxed regions. ORF 5-P1, P2, and P3 are specific for LDV-P. ORF 5-P4 is a mutated version of ORF 5-P3 with four amino acid substitutions. ORF 5-P5 represents the VP-3P ectodomain of LDV-C (Godeny *et al.,* 1993) and LDV-v (unpublished data). ORF 5-P6 contains combined amino acid substitutions found in the ectodomains of VP-3P of LDV-vx and LDV-a, another non-neuropathogenic LDV isolate provided by W. Murphy (unpublished data). ORF 5-P7 is a truncated form of LDV-P ORF 5-P3 with five amino acids removed from the N-terminus and four from the C-terminus. (B) Ψ designates the polylactosaminoglycan chains on the processed ectodomain of VP-3P of the non-neuropathogenic LDVs and the N- and C-terminal ends of the proteins are indicated. The -S-S- indicates the postulated position of the disulfide bond that bridges the ectodomains of VP-3P and M/VP-2 (Faaberg *et al.,* 1995; Faaberg and Plagemann, 1995).

neuropathogenic LDVs are very similar and strongly cross react.

RESULTS AND DISCUSSION

To identify the neutralization epitope(s) of LDV, we directed our attention primarily to VP-3P, since previous results from two laboratories showed that all anti-LDV neutralizing MAbs that have been generated so far are directed to VP-3P (Harty and Plagemann, 1988; Coutelier *et al.,* 1986; Coutelier and van Snick, 1988). Anti-VP-3P antibodies are also the primary antibodies generated in infected mice (Coutelier *et al.,* 1986; Cafruny *et al.,* 1986). VP-3P is disulfide bonded, probably via its short ectodomain (about 30 amino acids long) to the ectodomain of the non-glycosylated envelope protein, M/VP-2 (encoded by ORF 6; Faaberg *et al.,* 1995; Faaberg and Plagemann, 1995; see Fig. 1B). The membrane topography of VP-3P suggested that its neutralization epitope(s) is located in this ectodomain. To investigate this postulation we have used indirect ELISA to measure the binding of polyclonal anti-LDV antibodies (IMP), and also of various neutralizing and non-neutralizing MAbs, to a synthetic peptide representing the complete VP-3P ectodomain of LDV-P (ORF 5-P3; see Fig. 1A). Although virus neutralization epitopes are generally non-contiguous three dimensional structures, using a linear synthetic peptide seemed justified in the case of LDV VP-3P, since its processed ectodomain containing the postulated neutralization epitope is very short and cross linkage to M/VP-2 must impose certain structural constraints. We also assessed the binding of the various antibodies to synthetic peptides representing other hydrophilic segments of VP-3P and of other LDV-proteins (see Fig. 1A and Materials and Methods).

The results in Fig. 2A show that the ORF 5-P3 peptide representing the entire VP-3P ectodomain of LDV-P specifically bound antibodies from the plasma of FVB mice that had been infected with LDV-PLA (consisting of non-neuropathogenic LDV-P and LDV-vx) for 3 months. The IMP did not significantly react with the peptides representing the N-terminal end of the VP-3P ectodomain (ORF 5-P1) or a C-terminal segment of VP-3P (ORF 5-P2). Furthermore, substitution of four amino acids in ORF 5-P3 (ORF 5-P4) resulted in almost complete loss of reactivity with IMP. Antibodies generated in mice infected with the neuropathogenic LDV-C or LDV-v also reacted with the LDV-P specific ORF 5-P3 (Fig. 2B). The results indicate that IMP from both LDV-PLA and LDV-C/v infected mice contained antibodies that specifically reacted with the ectodomain of VP-3P of LDV-P.

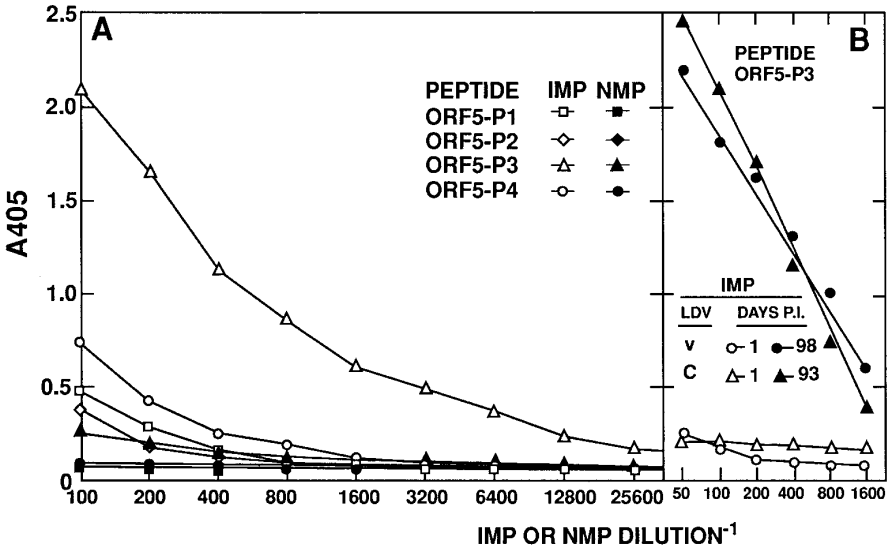

FIG. 2. ELISA measurements of the binding of NMP and IMP from LDV-PLA infected mice to different peptides of the ORF 5 protein (A) and of IMP from LDV-C and LDV-v infected mice to ORF 5-P3 (B). (A) ORF 5-P1–P4 are synthetic peptides representing different hydrophilic segments of the ORF 5 protein of LDV-P (see Fig. 1A). IMP was collected from a group of 3-month LDV-PLA infected mice. NMP was collected from mice that had not been exposed to LDV. ELISA was conducted as described under Materials and Methods. (B) IMP was collected from two or three FVB mice at the indicated times p.i. with cloned LDV-C or LDV-v.

That the ectodomain of VP-3P contains a neutralization epitope was unequivocally proven by the specific binding of neutralizing MAbs to the peptide ORF 5-P3. The neutralizing MAb 159-19 (see Table 1) strongly bound to ORF 5-P3 representing the ectodomain of LDV-P VP-3P, but not to ORF 5-P1 or ORF 5-P2 (Fig. 3). As with IMP, the four amino acid substitutions in ORF 5-P3 (ORF 5-P4) abolished the binding of MAb 159-19. There was also no interaction of MAb 159-19 with the synthetic peptides derived from segments of the nucleocapsid protein (N/VP-1), the non-glycosylated envelope protein (M/VP-2) or the ORF 3 protein (data not shown). Furthermore, a non-neutralizing MAb to VP-3P (159-4) reacted only very weakly with ORF 5-P3 and not with any of the other ORF 5 peptides (Fig. 3 and data not shown). Thus, the reaction of the neutralizing MAb 159-19 with the VP-3P ectodomain (ORF 5-P3) was highly specific. All other neutralizing MAbs that have been shown in competitive ELISA analyses to react with the same epitope as MAb 159-19, namely 159-7, 159-12, 159-16 and 159-18 (Harty and Plagemann, 1988; see Table 1), exhibited the same high specificity for ORF 5-P3 (data not shown). In fact, ascites fluids of some of these MAbs possessed titers for ORF 5-P3 of over 100,000 (see later, Fig. 5). Similarly, a number of neutralizing MAbs to VP-3P generated in another laboratory (E7, H12, H9 and A4; Coutelier and van Snick, 1988) were specific for ORF 5-P3 (Fig. 4). In contrast, none of the peptides reacted significantly with four other non-neutralizing MAbs to VP-3P, namely 159-3, 159-5, 159-13 and 159-14 (Table 1) that recognize at least two different epitopes on VP-3P (Harty and Plagemann, 1988; data not shown), or with MAbs to N/VP-1 (C3502017) or

TABLE 1

Characteristics of Anti-LDV MAbs

MAb	Isotype	Specificity	Neutralization[a]
159-7[b]	IgG2a	VP-3P	+
159-12	IgG2b	VP-3P	+
159-16	IgG2b	VP-3P	+
159-18	IgG2a	VP-3P	+
159-19	IgG1	VP-3P	+
159-3	IgG1	VP-3P	−
159-4	IgG1	VP-3P	−
159-5	IgG1	VP-3P	−
159-13	IgG1	VP-3P	−
159-14	IgG1	VP-3P	−
B6503-E7[c]	IgG2a	VP-3P	+
C3904-H12[c]	IgG3	VP-3P	+
B6505-H9[c]	IgG2a	VP-3P	+
B6501-A4[c]	IgG2a	VP-3P	+
F2[d]	IgG1	ORF 3 protein	−
C350201.7[c]	IgG1	N/VP-1	−

[a] In vitro neutralization of LDV-PLA, LDV-RIL, LDV-P, LDV-C, LDV-v, or all of them (Harty and Plagemann, 1988; Coutelier and van Snick, 1988; and unpublished data). In vitro neutralization of LDV infectivity was measured as described previously (Coutelier *et al.,* 1986; Cafruny *et al.,* 1986; Plagemann *et al.,* 1992). In brief, samples of LDV were incubated with NMP or anti-LDV antibodies in vitro and residual infectivity was measured by an end point dilution assay in mice.

[b] The battery of MAbs 159 was generated to formalin-inactivated LDV-PLA (Harty and Plagemann, 1988).

[c] These MAbs were generated with spleen cells from LDV-RIL infected mice, except for C3502017 which was generated to isolated nucleocapsids (Coutelier *et al.,* 1986; Coutelier and van Snick, 1988). They were provided by J.-P. Coutelier.

[d] MAb F2 was generated with spleen cells from LDV-PLA infected mice (unpublished data).

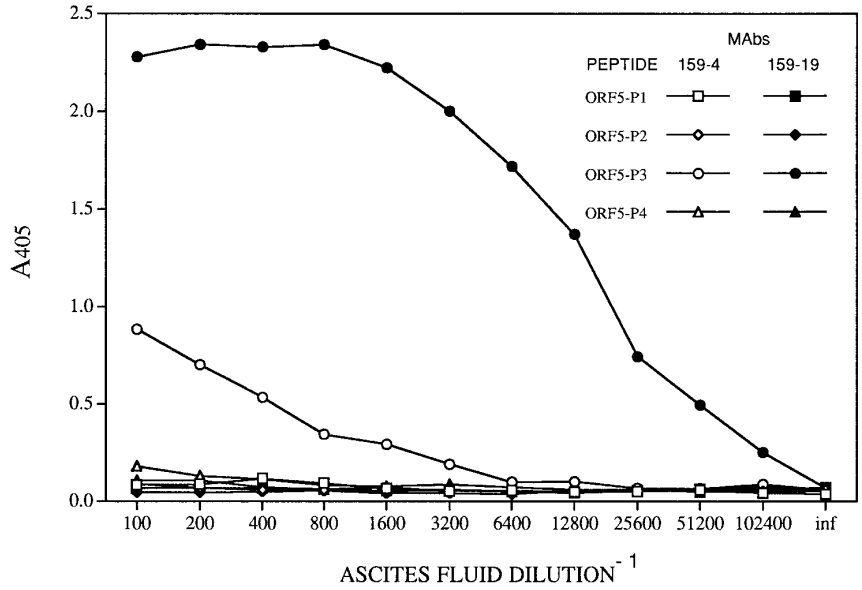

FIG. 3. ELISA measurements of the binding of neutralizing (159-19) and non-neutralizing (159-4) anti-VP-3P MAbs to peptides ORF 5-P1-P4. MAbs 159-19 and 159-4 were generated to formalin-inactivated LDV-PLA (Harty and Plagemann, 1988; see Table 1) and used in ascites fluid prepared in BALB/c mice. ORF 5-P1–P4 represent different segments in the VP-3P of LDV-P (see Fig. 1A). ELISA was performed as described under Materials and Methods.

to the nonstructural glycoprotein encoded by ORF 3 (F2; Fig. 4).

To further investigate the nature of the neutralization epitope, we have assayed the MAb reactivity of a peptide that represented the ectodomain of the neuropathogenic LDV-C and LDV-v (ORF 5-P5; see Fig. 1A). We also tested a peptide, ORF 5-P6, that differed from the LDV-P specific ORF 5-P3 by three amino acids that are present in the VP-3P ectodomains of LDV-vx, of LDV-a (another non-neuropathogenic LDV isolate) or both. Both peptides bound neutralizing MAb 159-18 to a similar extent as ORF 5-P3 (Fig. 5), although the reactivity of the LDV-C/v specific ORF 5-P5 was slightly lower than those of the LDV-P and LDV-vx/a specific peptides (ORF 5-P3 and ORF 5-P6, respectively). The results clearly indicate that the neutralization epitopes of the five LDV quasispecies

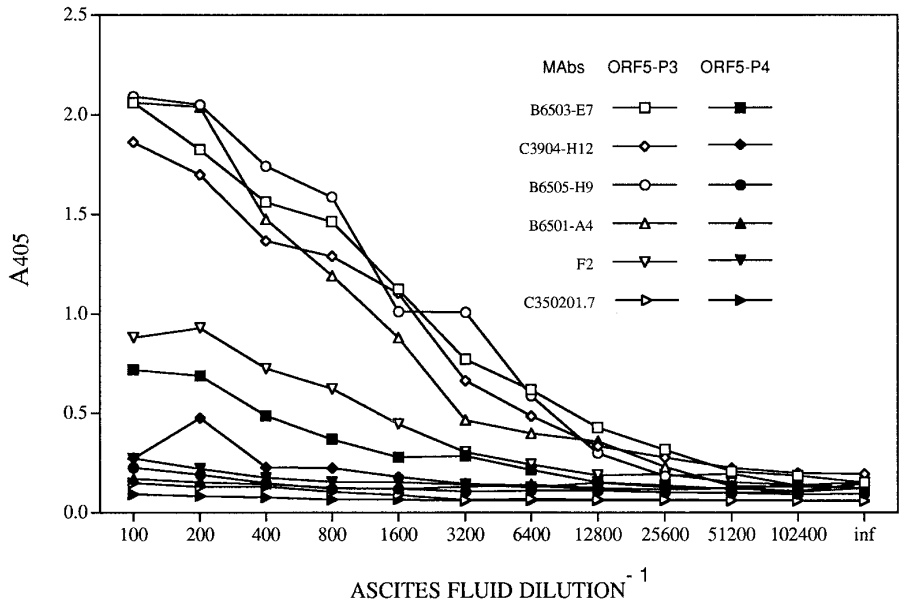

FIG. 4. ELISA measurements of the binding of additional anti-VP-3P neutralizing MAbs (B6503-E7, C3904-H12, B6505-H9, and B6501-A4), an anti-ORF 3 protein MAb (F2) and an anti-N/VP-1 MAb (C350201.7) to ORF 5-P3 and P4. The origins and properties of the MAbs are described in Table 1 ORF 5-P3 represents the VP-3P ectodomain of LDV-P, whereas ORF 5-P4 is a mutated ORF 5-P3 with four amino acid substitutions (see Fig. 1A).

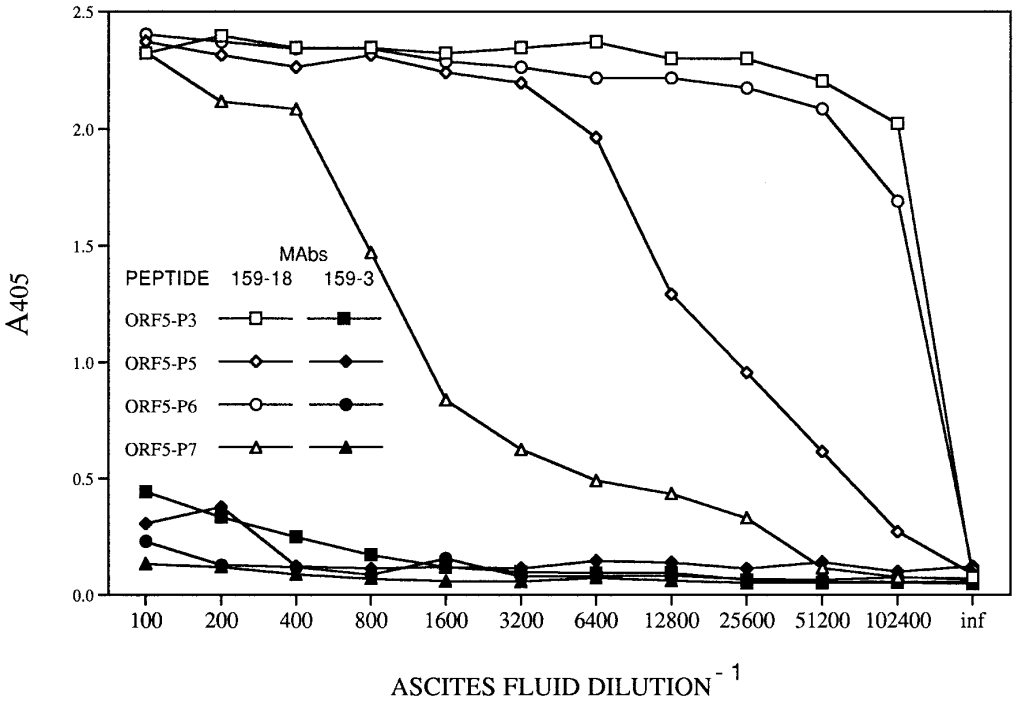

FIG. 5. ELISA measurements of the binding of neutralizing MAb 159-18 and non-neutralizing MAb 159-3 (see Table 1) to peptides representing the VP-3P ectodomain of LDV-P (ORF 5-P3), LDV-C/v (ORF 5-P5), and LDV-vx/a (ORF 5-P6) and to a truncated form of LDV-P ORF 5-P3 (ORF 5-P7).

are very similar with respect to the binding of neutralizing antibodies. In order to more clearly define the neutralization epitope in the VP-3P ectodomain, we measured the binding of the MAbs to a truncated version of ORF 5-P3 (ORF 5-P7) in which five amino acids from the N-terminus and four amino acids from the C-terminus were omitted (see Fig. 1A). This peptide specifically bound neutralizing MAb 159-18, but less efficiently than did ORF 5-P3, P5 or P6 (Fig. 5). The results indicate that the truncated peptide contains the major part of the neutralization epitope, but may be missing amino acids required for optimal antibody binding. If this is the case, the missing amino acids may be located at the C-terminal end since amino acid substitutions in the N-terminal region did not seem to affect antibody binding efficiency. However, I^{43}, Y^{44} or both seem critical for neutralizing antibody binding, since substitution by T and S, respectively, abolished all binding activity. In contrast, the same amino acid substitutions at residues 45 and 46, as in the LDV-C/v epitope (see Fig. 1A), affected binding only minimally (Fig. 5). That the neutralization epitope is located in the ectodomain between amino acids 37 and 60 is also indicated by our sequence analysis of ORF 5 from a neutralization escape variant previously isolated from LDV-PLA (Harty and Plagemann, 1988). The variant was completely resistant to in vitro neutralization by MAbs 159-12 and 159-18 (Harty and Plagemann, 1988). ORF 5 of this variant was found to differ from that of LDV-P by only two nucleotides (data not shown) resulting in one amino acid substitution in its VP-3P, $T^{39} \rightarrow A$, which was located in the neutralization epitope and thus probably responsible for the increased resistance to antibody neutralization.

In summary, we have demonstrated, by indirect ELISA using synthetic peptides, that the neutralization epitope of LDV is located on the short ectodomain of VP-3P. The ELISA using a synthetic peptide containing the neutralization epitope provides a novel method for measuring LDV neutralizing antibodies which is highly specific, rapid, inexpensive and much more sensitive than the assay based on the in vitro neutralization of infectious virions. Also, it does not require mice for quantitation of non-neutralized virions. With this method we have demonstrated that MAbs to the non-neuropathogenic LDVs bind about equally to the epitopes of the neuropathogenic and non-neuropathogenic LDV quasispecies (Table 1 and Fig. 5). Vice versa, antibodies generated in neuropathogenic LDV-C/v infected mice bind efficiently to the neutralization epitope of non-neuropathogenic LDV-P (Fig. 2B). The results indicate strong cross-reactions between all LDV quasispecies identified so far and that the amino acid differences in their neutralization epitopes do not greatly affect the binding of neutralizing antibodies. In contrast, a slight shifting in the position of four amino acids in the neutralization epitope specific for LDV-C/v (V^{33}, D^{36}, T^{45}, S^{46} to V^{32}, D^{35}, T^{43}, S^{44}), almost completely abolished antibody binding to the epitope (Figs. 2A, 3, and 4). Since the N-terminal amino acids did not seem to affect antibody binding efficiency, the I^{43}, Y^{44},

or both must be important for recognition by neutralizing antibodies.

It is unusual that the LDV neutralization epitope appears to be a linear peptide, since antibodies tend to recognize conformational structures that are often noncontiguous. The linear nature of the epitope may be due to the shortness of the processed ectodomain of VP-3P and its disulfide bonding to the even shorter (about 11 amino acid long) ectodomain of M/VP-2 (see Fig. 1A). This structure may also limit the number of potential epitopes on the virion surface. Our results also indicate that the LDV neutralization epitope is an unmodified peptide, whereas in VP-3P of virions it is associated with three or one polylactosaminoglycan chain(s) for the non-neuropathogenic and neuropathogenic LDVs, respectively (see Fig. 1A). It seems possible therefore that the generation of neutralizing antibodies by non-neuropathogenic LDVs depends on the removal of one or more of the polylactosaminoglycan chains leading to exposure of the epitope. This suggestion is supported by our recent finding that neutralizing antibodies are generated more rapidly in neuropathogenic than non-neuropathogenic LDV infected mice (unpublished data).

The finding that the neutralizing epitopes of neuropathogenic and non-neuropathogenic LDVs are serologically very similar is important in relation to the impaired ability of the neuropathogenic LDVs to establish a high viremic persistent infection (Chen *et al.,* 1997; and unpublished data). Recent studies in progress suggest that this impaired ability of the neuropathogenic LDVs to establish a persistent infection is partly due to increased immunogenicity but more importantly to an increased sensitivity to neutralization by antibodies (unpublished data). The finding that the neutralization epitopes of the neuropathogenic and non-neuropathogenic LDVs are closely related and cross react, suggests that differences in sensitivity to antibody neutralization are most likely not due to amino acid differences in their neutralization epitopes per se but rather to differences in the number of polylactosaminoglycan chains associated with their neutralization epitopes (see Fig. 1A).

The presence of neutralization epitope(s) on the ectodomain of the primary envelope glycoprotein encoded by ORF 5 may be a common characteristic of arteriviruses. In the case of equine arteritis virus (EAV), three overlapping neutralization epitopes are also located in the ectodomain of the primary envelope glycoprotein (G_L) just upstream of the first transmembrane segment (Balasuriya *et al.,* 1995). However, the ectodomain of the EAV G_L protein is much larger than that of VP-3P of LDV. On the other hand, the ORF 5 protein of the arterivirus porcine reproductive and respiratory syndrome virus is very similar to VP-3P of LDV in size, structure and amino acid sequence (about 50% amino acid identity; Plagemann, 1996). The protein also possesses neutralization epitopes but their location in the protein have not been identified (Pirzadeh and Dea, 1997).

MATERIALS AND METHODS

LDV

LDV concentrations were measured by an end point dilution assay in mice which is based on the plasma LDH elevation characteristic for LDV infected mice (Plagemann *et al.,* 1963). Virus titers were expressed as 50% infectious units (ID_{50}). Stocks of LDVs consisted of plasma harvested from groups of mice at 1 day p.i. and contained 10^9 to 10^{10} ID_{50}/ml. For long-term infections mice were infected by intraperitoneal injection of about 10^6 ID_{50}. To obtain plasma the mice were bled by the orbital method using heparinized Natelson blood collecting tubes (Chen and Plagemann, 1997).

Anti-LDV antibodies

Immune mouse plasma (IMP), containing polyclonal anti-LDV antibodies, was pooled from mice which had been infected with LDV for varying lengths of time (Cafruny *et al.,* 1986). The anti-LDV antibodies are primarily of IgG2a, IgG2b and IgG3 isotype and directed to the primary envelope glycoprotein (VP-3P), the nucleocapsid protein (N/VP-1) and the ORF 3 protein (Cafruny *et al.,* 1986; Coutelier *et al.,* 1986; Faaberg and Plagemann, 1997). IMP was freed of infectious LDV by three consecutive extractions with ethyl ether (Cafruny *et al.,* 1986). The anti-LDV monoclonal antibodies (MAbs) used in the present study and their properties are listed in Table 1. They were used in ascites fluid or after purification by protein A chromatography in the case of B6505-H9 and B6501-A4 (Coutelier and van Snick, 1988).

Measurement by ELISA of anti-LDV antibody binding to LDV peptides

Synthetic peptides representing various hydrophilic segments of LDV proteins were provided by the Biotechnology Core Facility at the Centers for Disease Control and Prevention. The ORF 5 protein (VP-3P) specific peptides (P1-P7) are outlined in Fig. 1A. Peptides representing segments of other LDV-P proteins were as follows: ORF 3 protein (a nonstructural glycoprotein; Faaberg and Plagemann, 1997), P1 = $L^{32} - G^{40}$, P2 = $F^{68} - S^{78}$; ORF 6 protein (the non-glycosylated envelope protein, M/VP-2), P1 = $A^{107} - G^{117}$, P2 = $V^{151} - G^{161}$; ORF 7 protein (nucleocapsid protein, N/VP-1), P1 = $S^2 - G^{13}$, P2 = $N^{32} - P^{43}$ (Palmer *et al.,* 1995; GenBank accession number U15146; the letters with superscript designations represent the starting and ending amino acids of each peptide).

Flat bottom, 96-well microtiter plates (Becton Dickinson, Lincoln Park, NJ) were used for indirect ELISA. The wells were coated overnight with the various synthetic

peptides in 25 mM carbonate-bicarbonate buffer, pH 9.6 (2 μg/well). The wells were washed five times with PBS containing 0.05% (v/v) Tween 20 (PBS-Tween) and then incubated with 100 μl of PBS-Tween containing 1% (w/v) bovine serum albumin (blocking solution) for 2 h at 37°C. The wells were again washed five times with PBS-Tween and then incubated with 100 μl of 2 fold serial dilutions made in blocking solution of either normal mouse plasma (NMP), IMP, or ascites fluid containing an anti-LDV MAb for 2 h at 37°C. After another set of washes with PBS-Tween, the wells were incubated with 100 μl of a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma, St. Louis, MO) for 1 h at 37°C. After five washes with PBS-Tween and two with Tris-HCl-buffered saline (pH 7.4), the wells were incubated with alkaline phosphatase substrate (Sigma) for about 30 min in the dark. The absorbency of the reaction solution was measured at 405 nm (A_{405}) with an automated microplate reader.

ACKNOWLEDGMENTS

We thank R. M. Wohlhueter for collaboration with the synthesis of the LDV specific peptides, J.-P. Coutelier for providing anti-LDV MAbs, and Brent Langley for excellent secretarial assistance. K.L. was supported by a graduate school research assistantship from the University of Minnesota. Z.C. was supported by USPHS training Grant CA 09138.

REFERENCES

Anderson, G. W., Even, C., Rowland, R. R. R., Palmer, G. A., Harty, J. T., and Plagemann, P. G. W. (1995a). C58 and AKR mice of all ages develop motor neuron disease after lactate dehydrogenase-elevating virus infection but only if antiviral immune responses are blocked by chemical or genetic means or as a result of old age. *J. Neurovirol.* 1, 244–252.

Anderson, G. W., Palmer, G. A., Rowland, R. R. R., Even, C., and Plagemann, P. G. W. (1995b). Infection of central nervous system glial cells by ecotropic murine leukemia virus in C58 and AKR mice and *in utero* infected CE/J mice predisposes mice to paralytic infection by lactate dehydrogenase-elevating virus. *J. Virol.* 69, 308–319.

Balasuriya, U. B. M., MacLachlan, N. J., de Vriess, A. A. F., Russitto, P. V., and Rottier, P. J. M. (1995). Identification of a neutralization site in the major envelope glycoprotein (G_L) of equine arteritis virus. *Virology* 207, 518–527.

Brinton-Darnell, M., and Plagemann, P. G. W. (1975). Structural and chemical-physical characteristics of lactate dehydrogenase-elevating virus and its RNA. *J. Virol.* 16, 420–433.

Cafruny, W. A., Chan, S. P. K., Harty, J. T., Yousefi, S., Kowalchyk, K., McDonald, D., Foreman, B., Budweg, G., and Plagemann, P. G. W. (1986). Antibody response of mice to lactate dehydrogenase-elevating virus during infection and immunization with inactivated virus. *Virus Res.* 5, 357–375.

Cavanagh, D. (1997). *Nidovirales:* A new order comprising *Coronaviridae* and *Arteriviridae. Arch. Virol.* 142/3, 629–633.

Chen, Z., and Plagemann, P. G. W. (1997). Detection of lactate dehydrogenase-elevating virus in transplantable mouse tumors by biological assay and RT-PCR assays and its removal from the tumor cells. *J. Virol. Meth.* 65, 227–236.

Chen, Z., Rowland, R. R. R., Anderson, G. W., and Plagemann, P. G. W. (1997). Coexistence in lactate dehydrogenase-elevating virus pools of variants that differ in neuropathogenicity and ability to establish a persistent infection. *J. Virol.* 71, 2913–2920.

Coutelier, J.-P. L., van Roost, E., Lambotte, P., and van Snick, J. (1986). The murine antibody response to lactate dehydrogenase-elevating virus. *J. Gen. Virol.* 67, 1099–1108.

Coutelier, J.-P. L., and van Snick, J. (1988). Neutralization and sensitization of lactate dehydrogenase-elevating virus with monoclonal antibodies. *J. Gen. Virol.* 69, 2097–2100.

Faaberg, K. S., and Plagemann, P. G. W. (1995). The envelope proteins of lactate dehydrogenase-elevating virus and their membrane topography. *Virology* 212, 512–525.

Faaberg, K. S., and Plagemann, P. G. W. (1997). ORF 3 of lactate dehydrogenase-elevating virus encodes a soluble, nonstructural, highly glycosylated, and antigenic protein. *Virology* 227, 245–261.

Faaberg, K. S., Even, C., Palmer, G. A., and Plagemann, P. G. W. (1995). Disulfide bonds between two envelope proteins of lactate dehydrogenase-elevating virus are essential for viral infectivity. *J. Virol.* 69, 613–617.

Godeny, E. K., Chen, L., Kumar, S. N., Methven, S. L., Koonin, E. V., and Brinton, M. A. (1993). Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV). *Virology* 194, 585–596.

Harty, J. T., and Plagemann, P. G. W. (1988). Formalin inactivation of the lactate dehydrogenase-elevating virus reveals a major neutralizing epitope not recognized during natural infection. *J. Virol.* 62, 3210–3216.

Harty, J. T., and Plagemann, P. G. W. (1990). Monoclonal antibody protection from age-dependent poliomyelitis: Implications regarding the pathogenesis of lactate dehydrogenase-elevating virus. *J. Virol.* 64, 6257–6262.

Martinez, D., Brinton, M. D., Tachovsky, T. G., and Phelps, A. H. (1980). Identification of lactate dehydrogenase-elevating virus as etiological agent of genetically restricted, age-dependent polioencephalomyelitis of mice. *Infect. Immun.* 27, 979–987.

Murphy, W. H., Mazur, J. J., and Fulton, S. A. (1987). Animal model for motor neuron disease. *In* "Clinical Neuroimmunology," (W. M. H. Behan, P. O. Behan, and J. A. Aarli, Eds.), pp. 135–155. Blackwell Scientific Publications, Oxford, England.

Murphy, W. H., Nawrocki, J. F., and Pease, L. R. (1983). Age-dependent paralytic viral infection in C58 mice: Possible implication in human neurologic disease. *Prog. Brain Res.* 59, 291–303.

Palmer, G. A., Kuo, L., Chen, Z., Faaberg, K. S., and Plagemann, P. G. W. (1995). Sequence of genome of lactate dehydrogenase-elevating virus. Heterogeneity between strains P and C. *Virology* 209, 637–642.

Pirzadeh, B., and Dea, S. (1997). Monoclonal antibodies to the ORF 5 product of porcine reproductive and respiratory syndrome virus define linear neutralizing determinants. *J. Gen. Virol.* 78, 1867–1873.

Plagemann, P. G. W. (1996). Lactate dehydrogenase-elevating virus and related viruses. *In* "Virology," (B. N. Fields, D. M. Knipe, and P. M. Howley, Eds.), 3rd ed. pp. 1105–1120. Raven Press, New York.

Plagemann, P. G. W., Gregory, K. F., Swim, H. E., and Chan, K. K. W. (1963). Plasma lactic dehydrogenase elevating agent of mice: Distribution in tissues and effect on lactic dehydrogenase isozyme patterns. *Can. J. Microbiol.* 9, 75–86.

Plagemann, P. G. W., Harty, J. T., and Even, C. (1992). Mode of neutralization of lactate dehydrogenase-elevating virus by polyclonal and monoclonal antibodies. *Arch. Virol.* 123, 89–100.

Plagemann, P. G. W., and Moennig, V. (1992). Lactate dehydrogenase-elevating virus, equine arteritis virus and simian hemorrhagic fever virus: A new group of positive-strand RNA viruses. *Adv. Virus Res.* 41, 99–192.

Plagemann, P. G. W., Rowland, R. R. R., Even, C., and Faaberg, K. S. (1995). Lactate dehydrogenase-elevating virus—An ideal persistent virus? *Semin. Immunopathol.* 17, 167–186.

Riley, V., Lilly, F., Huerto, E., and Bardell, D. (1960). Transmissible agent associated with 26 types of experimental neoplasms. *Science* 132, 545–547.