N-Glycans on the short ectodomain of the primary envelope glycoprotein play a major role in the polyclonal activation of B cells by lactate dehydrogenase-elevating virus

Peter G. W. Plagemann,1 Quentin A. Jones2 and William A. Cafruny2

1 Department of Microbiology, University of Minnesota, Minneapolis, MN 55455, USA
2 Division of Basic Biomedical Sciences, Molecular Microbiology & Immunology Group, University of South Dakota School of Medicine, Vermillion, SD 57069, USA

The common biologically cloned isolates of lactate dehydrogenase-elevating virus (LDV-P and LDV-vx) invariably cause a polyclonal activation of B cells in immunocompetent mice. It is recognized by an at least 10-fold increase in plasma IgG2a levels and the de novo formation of immune complexes that most likely consist of autoantibodies and their antigens. The present study indicates that three closely spaced N-glycans on the short ectodomain of the primary envelope glycoprotein, VP-3P, of LDV-P/vx, play a major role in inducing the polyclonal proliferation of B cells. IFN-γ then seems to mediate the differentiation of the activated B cells to IgG2a-producing plasma cells. These conclusions are based on the finding that the IgG2a hypergammaglobulinaemia and immune complex formation were much lower in mice that were infected with LDV variants (LDV-C and LDV-v) whose VP-3P ectodomains lack two of the three N-glycans than in LDV-P/vx infected mice. In contrast, the VP-3P ectodomains of three neutralization escape variants of LDV-C/v whose VP-3P ectodomains possess three N-glycosylation sites caused a polyclonal activation of B cells comparable to that of LDV-P/vx.

Introduction

A polyclonal activation of B cells resulting in a marked elevation in plasma IgG, mainly of the IgG2a isotype, and the generation of autoantibodies is associated with acute infections by various viruses, but the mechanisms involved are not fully understood and appear to differ for different viruses (Ahmed & Oldstone, 1984; Coutelier et al., 1988). A prime example of a virus causing a persistent hypergammaglobulinaemia and autoantibody formation is the mouse arterivirus lactate dehydrogenase-elevating virus (LDV) (Notkins et al., 1966; Coutelier & van Snick, 1985; Cafruny et al., 1986a; Li et al., 1990; Plagemann et al., 1995). Plasma IgG2a levels increase from generally below 0–5 mg/ml to 2–6 mg/ml by 2 weeks post-infection (p.i.) with LDV (Li et al., 1990).

Previous studies have shown that the hypergammaglobulinaemia caused by LDV is only partly dependent on T cells. It occurs to some extent in nude and T cell-depleted mice (Li et al., 1990; Coutelier et al., 1990; Hu et al., 1992) and in the complete absence of an anti-LDV antibody response (Rowland et al., 1994; Cafruny et al., 1999). Thus it is not simply an indirect result of the antiviral immune response (bystander effect; Ahmed & Oldstone, 1984). It has been shown that B cells from T cell-depleted and non-depleted mice after an LDV infection exhibit a similar elevated proliferative response in vitro, but that the former do not produce IgG2a in vitro unless treated with lipopolysaccharide and IFN-γ (Coutelier et al., 1990). Furthermore, T cells from LDV-infected mice as early as 4 days p.i. produce IFN-γ much more rapidly and to higher levels in vitro after exposure to concanavalin A than T cells from uninfected mice, and IFN-γ mRNA is detected in the spleen (Plagemann et al., 1995). The results suggested that an LDV protein may function as a direct B cell mitogen and that the generation of IgG2a-producing plasma cells is then mediated by IFN-γ produced by T cells largely generated in the course of the anti-LDV immune response (Plagemann et al., 1995).

One possibility was that the B cell mitogenic response was mediated by the large N-glycans associated with the ecto-
domain of the primary envelope glycoprotein (VP-3) of LDV (see Fig. 1). We have therefore compared the increase in plasma IgG2a and formation of immune complexes in mice that are infected either with the two biologically cloned common quasispecies of LDV (LDV-P and LDV-vx) or with two biologically cloned laboratory mutants, LDV-C and LDV-v, that are neuropathogenic in C58 and AKR mice. In LDV-P/vx virions the VP-3 ectodomain possesses three N-glycosylation sites (Fig. 1) that carry large N-glycan chains (Faaberg & Plagemann, 1995). These N-glycans suppress the immunogenicity of the single LDV neutralization epitope that is located in the VP-3 ectodomain (Li et al., 1998) and render these LDVs susceptible to antibody neutralization (Chen et al., 2000). The VP-3 ectodomains of the neuropathogenic LDV-C/v lack the two N-terminal N-glycosylation sites (Fig. 1). This renders the immunogenicity of the neutralization epitope and renders these LDVs susceptible to antibody neutralization, but at the same time endows them with neuropathogenicity for C58/AKR mice (Chen et al., 1999, 2000). In addition, we examined IgG2a hypergammaglobulinaemia and the formation of immune complexes in mice infected with neutralization escape variants of LDV-C/v whose VP-3 ectodomains have regained two N-glycosylation sites (Fig. 1).

Methods

Mice. FVB mice were provided by the transgenic mouse facility of the University of Minnesota Medical School and wild-type and B cell-deficient C57BL/6 and SCID, nude and wild-type BALB/c mice were purchased from Jackson Laboratories. All mice were 4–6 weeks of age. Mice were bled by the orbital method using heparinated Natleson blood collection tubes (Chen & Plagemann, 1997).

LDVs. The non-neuropathogenic LDV-P and LDV-vx and the neuropathogenic LDV-C and LDV-v have been cloned out from original isolates LDV-PLA, LDV-C-BR and LDV-VIR (see Chen & Plagemann, 1997) by repeated end-point dilution in mice (Chen et al., 1997, 1998). LDV-74 is an LDV isolated from a wild house-mouse in Montana (Li et al., 2000). Stocks of these LDVs consisted of plasma harvested from groups of mice 1 day p.i. and contained approximately 10⁸ infectious dose (ID)₉₀/ml. Virus titres were determined by an end-point dilution assay in FVB mice that is based on the 5- to 10-fold plasma elevation of lactate dehydrogenase activity characteristic for LDV-infected mice (Chen & Plagemann, 1997).

Mice were infected intraperitoneally with about 10⁴ ID₉₀ of the LDV quasispecies indicated in appropriate experiments. In order to allow valid comparisons of mice infected with the various LDVs, we injected in general in an experiment groups of two mice with at least one neuropathogenic and one non-neuropathogenic LDV; the groups were then housed in separate cages.

Plasma IgG2a quantification. IgG2a was quantified by capture ELISA basically as described previously (Li et al., 1990). In brief, 96-well ELISA plates (Pro-Bind Assay Plate, Falcon 3915; Becton Dickinson) were coated with goat anti-mouse IgG (Sigma; 2 µg/ml in bicarbonate buffer, pH 9.6; 100 µl per well). After overnight incubation at room temperature, the plates were washed three times with PBS containing 0.05% (v/v) Tween 20 (PBS–Tween) and then incubated for 1 h at 37 °C with PBS–Tween containing 1% (v/v) BSA (blocking solution; 100 µl per well). Then the plates were incubated at 37 °C for 2 h with 100 µl per well of 2-fold dilutions of standard IgG2a (Southern Biotechnology; from 1 µg/ml to 0.5 ng/ml) or six 2-fold dilutions of plasma to be assayed (from 1:4000 to 1:128 000). The plates were washed a further three times with PBS–Tween and then incubated with alkaline phosphatase-conjugated goat anti-mouse IgG2a (Southern Biotechnology) diluted 1:1000 in blocking solution (100 µl per well). Thereafter the plates were washed three times with PBS–Tween and once with 50 mM Tris–HCl (pH 7.4) and incubated with alkaline phosphatase substrate (Sigma 104; 200 µl per well) for 30 min. The absorbance of the reaction product was measured at 405 nm with an automatic plate reader. The concentration of IgG2a of experimental samples was estimated by extrapolation from the linear portion of the standard curve. There was no cross-reaction with other IgG isotypes (Li et al., 1990). In general, two plates were processed at the same time allowing direct comparison of plasma samples from seven mice (four time-points each; see Fig. 2).

Quantification of immune complexes that directly bind to ELISA plates in PBS–Tween. The procedure was as described previously (Cafruny et al., 1986b; Hu et al., 1992). In brief, 96-well ELISA plates were coated with six 2-fold dilutions of each plasma sample in PBS–Tween (100 µl per well from 1:50 to 1:1600). After overnight incubation at room temperature, the plates were washed three times with PBS–Tween and then incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma A3562; 1:1000) in PBS–Tween (150 µl per well) for 1 h at 37 °C. The plates were then washed and developed with
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alkaline phosphatase substrate as already described above, except that the incubation was extended to 1 h or longer as indicated in appropriate experiments. Again, generally two plates were processed at the same time allowing the direct comparison of plasma samples from eight mice (four time-points each; see Fig. 4).

Results

In earlier studies of the polyclonal activation of B cells by LDV in mice, the mice were infected with LDVs that had been originally isolated as contaminants from mice carrying different transplantable mouse tumours (LDV-PLA and LDV-RIL; Li et al., 1990; Coutelier et al., 1990). These and other original LDV isolates have been found to be mixtures of two slightly different quasispecies, designated LDV-P and LDV-vx (Chen & Plagemann, 1997; Chen et al., 1998), which have been biologically cloned in mice (Chen et al., 1997, 1998). In the present study, we have compared plasma IgG2a increases in mice infected with the cloned LDV-P and LDV-vx and with the cloned neuropathogenic laboratory mutants LDV-C and LDV-v (Fig. 2). Each frame in Fig. 2 shows results for single or duplicate mice infected with various LDVs whose plasma samples were analysed in the same ELISA to allow a valid direct comparison. The results show that the cloned LDV-P and LDV-vx caused similar levels of hypergammaglobulinaemia in both C57BL/6 and FVB mice and similarly as previously reported for LDV-PLA (Li et al., 1990). On the other hand, the increase in plasma IgG2a was consistently only about one-half or less as high after infection of mice with the neuropathogenic LDV-C and LDV-v than after infection with LDV-P or LDV-vx (Fig. 2). Similar results were obtained with duplicate mice assayed in the same experiment (Fig. 2c, d), and with additional mice infected with these LDVs [at least two other FVB mice for each (data not shown) and two C57BL/6 mice (Cafruny et al., 1999, and data not shown)], and no increase in plasma IgG2a was observed in an uninfected companion mouse (see Fig. 2c), consistent with previous results (Li et al., 1990).

The absolute plasma IgG2a values estimated in the different experiments varied somewhat (Fig. 2). Those of uninfected C57BL/6 mice were generally lower (< 0.1 mg/ml) than those of uninfected FVB mice (0.1–0.5 mg/ml) and increased to a lower level than in FVB mice after an LDV infection (Fig. 2). Similar mouse strain differences in IgG2a levels have been observed previously (Li et al., 1990). In contrast, the observed slight variations in absolute IgG2a values for FVB mice in

Fig. 2. Time-courses of plasma IgG2a elevation in C57BL/6 and FVB mice after infection with LDV-P/vx (○, ●), LDV-C/v (▲, ▼) or LDV-74 (■) or that remained uninfected (◇). All time-courses are for single mice, but in (c) and (d) results for two companion mice are presented. Results in each frame come from a single ELISA, and were estimated from the same IgG2a standard curve. Comparable results were obtained in repeat ELISAs of the plasma samples (data not shown).

Fig. 3. Comparison of the time-courses of plasma IgG2a elevation in duplicate FVB mice infected with three neutralization escape mutants of LDV-v and LDV-C (a–c) with those in LDV-P- and LDV-C-infected mice (d). All values were obtained in the same ELISA and estimated from the same IgG2a standard curve. Comparable results were obtained in a repeat ELISA of the plasma samples (data not shown).
different experiments seem to be due to small variations (about 2-fold) in the standard IgG2a curves generated in different assays. In order to minimize such variations and to facilitate valid comparisons between the IgG2a levels in LDV-P/vx and LDV-C/v infected mice we have used the same stock solution of IgG2a for establishing the standard curves (1 µg to 0.5 ng/ml) in all assays and have simultaneously assayed the time-courses of IgG2a elevation (four time-points each; see Fig. 2) in seven mice infected with the two classes of LDV (analyzing 2-fold dilutions of plasma from 1:4000 to 1:28,000).

Since LDV-v is a genetic recombinant of LDV-vx that has, by a double recombination, specifically acquired the 5’ end of ORF5 of LDV-C (~ 400 nt), which encodes the ectodomain of the primary envelope glycoprotein VP-3P (Li et al., 1999), the difference in polyclonal B cell activation between LDV-P/vx and LDV-C/v, on the one hand, and LDV-C/v, on the other hand, must reside in this segment of VP-3P. The most likely molecular structure responsible for this difference in the polyclonal B cell activation is the number of N-glycans associated with the VP-3P ectodomain (Fig. 1), since there are no other amino acid differences in this VP-3P segment that correlate with the various differences in phenotypic properties of the two classes of LDV (Chen et al., 1998; Li et al., 1999).

This hypothesis was further explored by examining plasma IgG2a levels in FVB mice after infection with three neutralization escape mutants of LDV-C and LDV-v (LDV-v-T105/38, LDV-C-T168/55 and LDVC-T82/68N/76) whose VP-3P ectodomains had regained three N-glycosylation sites (see Fig. 1). The plasma IgG2a elevation in duplicate mice infected with each of the three neutralization escape mutants was comparable to that in an LDV-P infected mouse and much higher than that in an LDV-C infected mouse (Fig. 3). Comparable results were obtained in a repeat ELISA of the plasma samples; the results showed that the elevation in plasma IgG2a in the mice infected with all three neutralization escape mutants was similar to that in an LDV-vx-infected mouse and over twice as high as in an LDV-v-infected mouse (data not shown). Similarly, in a mouse infected with an LDV isolated from a wild house-mouse in Montana (LDV-74), whose VP-3P ectodomain possesses all three N-glycosylation sites (Li et al., 2000), plasma IgG2a levels increased similarly as in LDV-P- and LDV-vx-infected mice (Fig. 2).

An even more drastic difference between mice infected with the common non-neuropathogenic LDVs and the neuropathogenic mutants was observed in the formation of 150 to 300 kDa immune complexes that have been found to become generated concomitant with an LDV-induced polyclonal activation of B cells (Cafruny et al., 1986b; Hu et al., 1992). These complexes contain IgG2a, which most likely represents autoantibodies that are generated as a result of the polyclonal activation of B cells and bound to their autoantigens (Hu et al., 1992). They are recognized by binding in PBS–Tween to ELISA plates that are not coated with antigen. They differ in size and IgG isotype specificity from the infectious virion–antibody complexes that are also generated and persist in LDV-infected mice (Hu et al., 1992). Similar plate-binding immune complexes appear in MAIDS murine leukaemia virus-infected mice concomitant with a polyclonal activation of B cells (Even et al., 1992). Fig. 4(a–c) illustrates the appearance of plate-binding immune complexes in mice after infection with cloned LDV-P and LDV-vx. The time-courses of immune
complex formation were very similar for the two cloned quasispecies, just as was the case for the IgG2a hypergamma-globulinaemia induced by them (Fig. 2), and similar to those previously reported for mice infected with the original LDV-PLA isolate (Cafruny et al., 1986b; Hu et al., 1992). The immune complexes begin to appear between 7 and 14 days p.i. with LDV, concomitant with increases in plasma IgG2a, and reach maximum persistent levels by 3–5 weeks p.i. (Fig. 4a–c; see Cafruny et al., 1986b). No plate-binding immune complexes were detectable in the 1 day infected mice (Fig. 4). In contrast to the results with LDV-P/vx-infected mice, we detected little or no plate-binding IgG2a in the plasmas of FVB, C57BL/6 or CF1 mice infected with the neuropathogenic LDV-C or LDV-v in our standard assay, which involves a 1 h incubation of the

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**Fig. 5.** Comparison of the appearance of ELISA plate-binding activity in FVB mice infected with three neutralization escape mutants of LDV-v and LDV-C (a–c) with that in mice infected with LDV-P and LDV-vx and LDV-v (d–f) (T = time p.i. in days). All values were obtained in a single assay in which the ELISA plates were incubated with alkaline phosphatase substrates for 1–5 h. The results are for single mice, but comparable results were observed with duplicate mice which were infected with the neutralization escape mutants and assayed along with plasma samples from LDV-P- and LDV-C-infected mice (data not shown). Plasma samples of the same mice were also assayed for IgG2a levels (Fig. 3).

**Fig. 6.** Time-courses of viraemia of FVB mice infected with cloned LDV-P (●), LDV-vx (▲), LDV-C (○) or LDV-v (△) (a), and time-courses of viraemia of FVB mice infected with LDV-v at birth (b, ○) and levels of plasma IgG2a (b, ●) and of immune complexes (c, ▼, ▼) in these mice in comparison to those in mice infected with LDV-P when 28 days of age (b, ▲, c; ○, ●, △, ▲). (a) Time-courses of viraemia are for mice whose IgG2a plasma levels are shown in Fig. 2(e, f). In (b) five FVB pups of a litter were infected with LDV-v at birth and pools of their plasma obtained at the indicated times were assayed for infectious LDV and IgG2a levels (b) and for plate-binding immune complexes (c). In addition, two mice were infected with LDV-P when 28 days of age and their pooled plasma samples were assayed for IgG2a (b) and immune complexes (c). All Ig2a values were obtained in a single assay and this was also the case for the assay of immune complexes (1–5 h incubation with substrate).
ELISA plates with alkaline phosphatase substrate (Fig. 4e–h, and data not shown). However, a longer incubation of the ELISA plates with alkaline phosphatase substrate (3–4 h) revealed that some plate-binding immune complexes did become generated in mice infected with LDV-C (Fig. 4i) or LDV-v (data not shown). Repeated plate-binding comparisons under identical assay conditions indicated that the level of immune complexes generated in LDV-C/v infected mice represented 5–10% of those formed in LDV-P/vx-infected mice.

On the other hand, immune complex formation in mice infected with the three neutralization escape mutants of LDV-P and LDV-vx rather than to that in mice infected with LDV-P and LDV-vx rather than to that in mice infected with the LDV-C (or LDV-C) parent (Fig. 5). The same was the case for immune complex formation in a mouse infected with the wild house-mouse LDV-74 (Fig. 4d), whose VP-3P ectodomains possesses three N-glycosylation sites, just like those of LDV-P and LDV-vx.

Hypergammaglobulinaemia induction in LDV-infected mice is very rapid (within a few days; Cafruny et al., 1986a) just as is the humoral immune response to LDV (Cafruny et al., 1986b; Chen et al., 1999, 2000). Both seem to be induced by the large amounts of virus (up to $10^{10}$ ID$_{50}$/ml of plasma) produced during the first day p.i. which are generated by the productive infection of practically all LDV-permissive macrophages in the mouse (Plagemann, 1996). The amounts of infectious virus produced during the first day p.i. are about the same for LDV-P/vx and LDV-C/v (Fig. 6a; also see Chen et al., 1999, 2000). Thus, the low polyclonal activation of B cells by LDV-C/v does not seem to be due to low virus production. The time-courses of viraemia of LDV-P/vx and LDV-C/v start to diverge due to the selective neutralization of LDV-C/v only about 10 days p.i., i.e. at a time when the levels of viraemia for both classes of LDV have fallen already by 2 lg$_{10}$ (Fig. 6a) and the humoral immune response and polyclonal B cell activation have already been induced. The initial decrease in viraemia is due to physical inactivation since LDV is highly unstable at 37 °C (Plagemann, 1996).

That the low level of polyclonal activation of B cells by LDV-C/v is an intrinsic property of these LDVs is also indicated by an analysis of mice in which anti-LDV immune responses were specifically blocked by infection at birth (Rowland et al., 1984; Chen et al., 2000). Upon infection of newborn FVB mice, LDV-C/v established a persistent viraemia similar to those established by LDV-P/vx (cf. Fig. 6a, b; also see Chen et al., 2000). In spite of the high persistent viraemia in these tolerized mice (Fig. 6b), IgG2a hypergammaglobulinaemia was much lower than in mice infected with LDV-P at 28 days of age (Fig. 6b) and plate-binding immune complex formation was insignificant compared to that in the LDV-P/vx-infected mice (Fig. 6c). Results similar to those observed with LDV-v were obtained with FVB mice that were infected at birth with LDV-C (data not shown). In contrast, immune complex formation was comparable in FVB mice that were infected with LDV-P at birth or when 5 or 15 days of age (Rowland et al., 1994).

To further investigate the role of T and B cells in the polyclonal activation of B cells by an LDV infection, we compared the formation of plate-binding immune complexes in wild-type, nude and SCID BALB/c mice and in a B cell-defficient C57BL/6 mouse (Fig. 7). There was some immune complex formation in LDV-vx-infected nude BALB/c mice, but it was much lower than in LDV-vx-infected wild-type BALB/c mice (Fig. 7a, b), similar to that previously reported for LDV-PLA-infected nude Swiss mice and CD4+ T cell-depleted BALB/c mice and their wild-type companions (Hu et al., 1992). The degree of reduction in immune complex formation in nude and T cell-depleted mice correlated with a similar reduction in IgG2a hypergammaglobulinaemia (data not shown; Hu et al., 1992).
In contrast, no immune complexes were detected in LDV-P-infected SCID BALB/c mice or B cell-deficient C57BL/6 mice (Fig. 7c), and the plasmas of these mice were devoid of IgG2a (data not shown).

Discussion

Taken together, our results indicate that the three closely spaced N-glycans on the short ectodomain of the primary envelope glycoprotein VP-3P of LDV-P/vx virions play a major, if not primary, role in the polyclonal activation of B cells that is invariably associated with an acute infection of immunocompetent mice with these LDVs. This conclusion is indicated by the finding that LDV-C/v, whose VP-3P ectodomains lack two of the three N-glycans that are associated with the VP-3P ectodomain of LDV-P/vx (Fig. 1), induced a much lower IgG2a hypergammaglobulinaemia and immune complex formation than did the latter (Figs 2 and 4) and that this is an intrinsic property of these LDVs and not due to a lower virus production or to antibody neutralization (Fig. 6). Furthermore, three neutralization escape mutants of LDV-C/v whose VP-3P ectodomains had regained three N-glycans induced a polyclonal activation of B cells similar to that induced by LDV-P/vx (Figs 3 and 5) and this was also the case for an LDV isolated from a wild house-mouse, whose VP-3P ectodomain possesses all three N-glycosylation sites.

The finding that an LDV infection, even in CD4+ T cell-depleted mice, induces a general B cell proliferation (Coutelier et al., 1990) indicates that the B cell stimulation is occurring independently of T cell help. Thus, the VP-3P ectodomain with its N-glycans is a direct B cell mitogen behaving like a TI-1 antigen similar to bacterial polysaccharides, lipopolysaccharides and polymeric proteins (Janeway et al., 1999).

It is unclear, however, why the N-glycans on the VP-3P ectodomain play the major role in B cell activation, since the LDV genome encodes three other glycoproteins (Plagemann, 1996). ORF2 encodes a very minor envelope glycoprotein, whose about 160 amino acid long ectodomain also carries three closely spaced N-glycans (Faaberg & Plagemann, 1995). ORF 3 encodes a soluble non-structural glycoprotein with six widely spaced N-glycosylation sites and ORF 4 encodes a non-structural, membrane-associated, glycoprotein with unknown functions, whose ectodomain possesses five N-glycosylation sites (Faaberg & Plagemann, 1997). Perhaps the VP-3P ectodomain with its N-glycans exposed on the surface of LDV virions forms a rather rigid structure that is required for its B cell mitogenic activity. The VP-3P ectodomain is very short (about 30 amino acids long; see Fig. 1) and linked by a disulfide bond to the even shorter (about 11 amino acids long) ectodomain of the matrix (M) protein, probably via conserved cysteine residues in the ectodomains of the two proteins (Faaberg & Plagemann, 1995). In addition, or alternatively, B cell activation may require cross-linkage of receptor sites on the B cells, or at least multiple interactions between sites on the inducer and sites on a B cell, and such can only be accomplished by the high density of VP-3P ectodomains covering intact LDV virions. It is of interest that the polyclonal activation of B cells coincides with the accumulation of large numbers of virions in newly formed germinal centres of the spleen and lymph nodes (Anderson et al., 1995). If the N-glycans of the VP-3P ectodomain are solely responsible for the B cell mitogenic activity of LDV, one would predict that loss of all three N-glycosylation sites on VP-3P would abolish all B cell mitogenic activity of LDV. We have not been able to test this hypothesis since we have not been able to strip LDV virions of the N-glycans without loss of infectivity and have not found an LDV mutant that lacks all N-glycosylation sites in VP-3P. Thus, we cannot rule out that some other LDV glycoprotein may contribute to the B cell mitogenic activity of LDV.

The finding that a hypergammaglobulinaemia was induced by an acute LDV infection and not by injection of glutaraldehyde-inactivated LDV (Cafruny et al., 1986a) can be readily explained as a concentration difference. Polyclonal activation of B cells is only observed at relatively high concentrations of a TI-1 antigen (Janeway et al., 1999), which was probably not achieved by injection of the inactivated virus, whereas an acute infection results in the production of very large amounts of progeny virions during the first day p.i. (Fig. 6). Furthermore, inactivation of LDV by treatment with glutaraldehyde may destroy the structures on the virion surface that are critical for B cell activation as well as for the generation of neutralizing MAbs, since none of the anti-VP-3P MAbs generated to glutaraldehyde-inactivated LDV possessed neutralizing activity (Harty et al., 1987).

Although T cell help does not seem to be required for the induction of B cell proliferation by LDV, CD4+ T cells clearly play some role in the induction of IgG2a production since both the IgG2a hypergammaglobulinaemia and immune complex formation are greatly reduced in nude mice and CD4+ T cell-depleted mice (Hu et al., 1992; Fig. 7). As discussed already, it seems likely that Th1 cells generated in the course of the anti-LDV immune response of the host and induced by IL-12 produced by LDV-infected or activated macrophages (Coutelier et al., 1995) provide the IFN-γ that mediates the differentiation of the activated B cells to IgG2a-producing plasma cell. This view is further supported by the recent finding that both the IgG2a hypergammaglobulinaemia and autoantibody and immune complex formation are also reduced in C57BL/6 IFN-γ gene knock-out mice, whereas LDV viraemia was the same as in wild-type C57BL/6 mice (Cafruny et al., 1999).

Another finding that requires an explanation is that the formation of plate-binding immune complexes is reduced in LDV-C/v-infected mice, as compared to LDV-P/vx-infected mice, much more than the IgG2a hypergammaglobulinaemia (Figs 2 and 4). A similar finding has been made in comparing the polyclonal activation of B cells in LDV-P-infected IFN-γ gene knock-out and wild-type mice (Cafruny et al., 1999).
These results suggest that the increased production of polyclonal IgG2a does not necessarily lead to the generation of immune complexes containing IgG2a autoantibodies. An explanation may be derived from the fact that only a very minor fraction of the total IgG2a that is produced in LDV-infected mice is sequestered in immune complexes (Hu et al., 1992) as well as the likelihood that the autoantibodies in these complexes are produced by a subset of B cells, such as CD5 B cells (B1 cells; Janeway et al., 1999), that may respond differently to the mitogenic activity of LDV than the bulk of the B cells. On the other hand, it is possible that factors other than autoantibodies and their antigens are involved in the formation of the ELISA plate-binding immune complexes since we have not been able to artificially generate plate-binding immune complexes in vitro or to regenerate the immune complexes once they have been dissociated (Hu et al., 1992).

To our knowledge, this is the first report implicating N-glycans exposed on the surface of an enveloped virus in the polyclonal activation of B cells during an acute infection. It seems likely that other enveloped viruses that cause a polyclonal activation of B cells possess similar N-glycan-containing structures associated with their envelope glycoproteins that function in a similar manner.

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References


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