Infection of Mice with Lactate Dehydrogenase-elevating Virus Leads to Stimulation of Autoantibodies

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SUMMARY

The development of autoimmunity was investigated in BALB/c and C58 mice infected with lactate dehydrogenase-elevating virus (LDV). Autoantibodies reactive by ELISA with syngeneic central nervous system antigens appeared early during LDV infection of both strains of mice, and were maintained for many months. Western blot analysis indicated that the LDV-induced autoantibodies reacted with a variety of different brain antigens, and mouse strain differences in the pattern of autoreactivity were observed. LDV infection of C58 and BALB/c mice also stimulated antibodies reactive with syngeneic liver-, kidney- and spleen-derived antigens, and in Swiss outbred mice heart-reactive antibodies were observed following LDV infection. These results show that autoimmunity is a feature of the deregulation of the immune system which occurs during LDV infection.

Lactate dehydrogenase-elevating virus (LDV) is a persistent togavirus of mice (Rowson & Mahy, 1975). Although immunogenic (Notkins et al., 1968; Cafruny & Plagemann, 1982a; Cafruny et al., 1986c), the virus is never cleared from the host, and viral persistence is associated with various poorly understood effects on host immunity, including polyclonal activation of immunoglobulin synthesis (Cafruny & Plagemann, 1982a; Coutelier & Van Snick, 1985), and the presence of hydrophobic immune complexes in the circulation (Cafruny et al., 1986b). LDV is also the aetiological agent of fatal motor neuron disease in genetically susceptible C58 strain mice (Martinez et al., 1980; Nawrocki et al., 1980; Murphy et al., 1983). The pathogenesis of this disease is unknown, although it is influenced by viral, genetic and immunological factors (Murphy et al., 1983). The present study was undertaken to determine whether persistent LDV infection stimulates the production of autoantibodies. In BALB/c and C58 mice infected with LDV, antibodies which reacted by ELISA with normal mouse brain antigens were found to appear within 1 week post-infection (p.i.), and to remain detectable for up to 8 months p.i. Western blot analysis revealed different patterns of antigen reactivity between C58 and BALB/c mice during the early phase of LDV infection. In addition, autoantibodies reactive with heart-, kidney-, liver- and spleen-derived antigens were also found during LDV infection of mice. These results are significant in relation to the study of deregulated immunity during persistent viral infection, and may lead to a better understanding of how viruses modulate the host immune response.

This study began with a determination by ELISA of the levels of antibodies that bound to brain or spinal cord antigens, present in the blood of LDV-infected mice. Mice infected with LDV were bled at various times p.i. and their blood plasma was stored for future antibody analysis. Antigens were prepared from sonicated normal mouse tissue and added to the wells of ELISA plates. Serial dilutions of pooled blood plasma from persistently LDV-infected or control mice were then added to the antigen-coated plate wells, and bound immunoglobulin was measured by a standard ELISA technique. Results in Fig. 1(a) show that plasma from LDV-
infected BALB/c and C58 mice contained IgG anti-brain titres of about 1:800. IgG anti-spinal cord titres were also found to be about 1:800 (data not shown). We have also tested LDV-infected mice for antibodies reactive with other mouse tissue antigens and various heterologous antigens. Both C58 and BALB/c mice developed antibodies which reacted by ELISA with normal mouse kidney, liver and spleen antigens during the course of LDV infection, and outbred Swiss mice infected with LDV developed heart-reactive antibodies (Fig. 1b). In contrast, LDV infection did not stimulate antibodies that reacted with several non-mouse antigens (lipopolysaccharide of *Salmonella typhimurium*, bovine serum albumin, sheep erythrocytes), but antibodies which reacted by ELISA with rabbit γ-globulin were found after LDV infection (data not shown).

The time course of appearance of anti-brain antibodies was studied in BALB/c and C58 mice. Plasma samples from groups of six mice were pooled at various times p.i. with one of two strains of LDV [the neuroparalytic Murphy strain designated LDV*<sub>MUR</sub>*, or the relatively non-paralytic Plagemann strain designated LDV*<sub>PLA</sub>* (Cafruny & Plagemann, 1982b)] and 1:200 dilutions were tested by ELISA for reactivity with normal mouse brain antigens. Fig. 2 shows that anti-brain IgG appeared within 1 week of LDV infection and persisted throughout the 8 month period of study. Levels of IgG anti-brain antibodies reached a peak at about 2 weeks p.i., after which cyclic variations were apparent. IgM anti-brain antibodies showed similar cyclic variations (data not shown).

The next experiments were designed to characterize the brain antigens used and the nature of the anti-brain response in LDV-infected mice. PAGE of a brain antigen preparation from
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Fig. 2. Time course of appearance of anti-brain IgG. Groups of six C58 or BALB/c mice were intraperitoneally infected with about $10^6$ ID$_{50}$ LDV. The mice were bled weekly and plasma samples were pooled for each group and stored at $-70^\circ$C for future antibody analysis. Plasma samples diluted 1:200 in pH 7.4 PBS-Tween 20 were analysed for reactivity with C58 brain antigen by ELISA, essentially as described in Fig. 1. BALB/c, LDVMuR ($\Delta$); BALB/c LDVP LA ($\triangle$); C58, LDVMuR (○); C58, LDVP LA (○).

normal C58 mice, along with normal C58 mouse plasma, is presented in Fig. 3(a). The results show the presence of a broad range of protein bands in the brain preparation, and also demonstrate minimal contamination of the brain preparation with blood proteins, as exemplified by the relative absence of albumin. In order to determine the specificity of the anti-brain antibodies, Western blot experiments were carried out, using either pooled plasma samples or individual plasma samples from uninfected control and LDV-infected mice. In Western blot experiments using plasma samples obtained from mice between 2 and 3 weeks p.i., C58 strain but not BALB/c strain mice showed reactivity with normal mouse brain proteins (Fig. 3b, c). Plasma pooled from three C58 mice at 14 days p.i. contained IgG which reacted with a 48K protein present in normal C58 brain, and weak reactivity was also observed with proteins of 69K, 91K and 96K (Fig. 3b; reactivity with the latter three proteins was not strong enough to reproduce in the photograph). Plasma pooled from three BALB/c mice at 14 days p.i. or from 10 uninfected C58 mice (normal mouse plasma, N) failed to react detectably in the same experiment. In Fig. 3(c), results from experiments using individual plasma samples obtained from mice that had been treated with cyclophosphamide (200 mg/kg) prior to LDVMuR infection are shown. Eighteen to 21 days p.i. some of the C58 mice which developed fatal paralysis also demonstrated Western blot reactivity with normal C58 brain proteins of 70K, 80K, or 150K, while all BALB/c mice remained healthy under the same experimental conditions and were non-reactive by Western blot analysis. Of a total of 14 individual C58 mice studied at 2 to 3 weeks p.i., six (43%) were found to be positive by Western blot analysis for IgG anti-brain antibodies, while none of 10 BALB/c mice studied under the same conditions were found to be positive. This contrasts with the various amounts of IgG anti-brain antibodies which were detected by ELISA in all of the plasma samples obtained from LDV-infected mice regardless of the strain or prior drug treatment. Furthermore, during the later stages of chronic LDV infection, BALB/c as well as C58 mice were found to develop anti-brain IgG detectable by Western blotting, and many more protein bands became detectable, as revealed by the blot representing plasma pooled from about 50 mice at 8 months p.i. and enhanced by the avidin–biotin system (Fig. 3d). Western blot analyses failed to detect IgG in plasma from BALB/c mice 8 months p.i. that reacted with antigens present in normal BALB/c plasma (Fig. 3e), suggesting a cellular origin of the reactive antigens.
Fig. 3. Western blot analyses of mouse anti-brain IgG, and protein analysis of mouse brain antigen. (a) C58 mouse brain antigen preparation (Br) and normal mouse plasma (N) pooled from ten C58 mice were separated by SDS–PAGE and the protein bands were stained with Coomassie Brilliant Blue. Total protein content of the loaded samples was between 60 to 90 μg. (b to d) Western blot analyses of IgG anti-C58 brain antibodies present in blood plasma from LDV-infected C58 or BALB/c mice or in control plasma obtained from uninfected C58 mice. Following PAGE of C58 brain antigen, the proteins were blotted onto nitrocellulose strips, which were then cut out and incubated with 1:100 dilutions of the sources of primary antibody indicated (C58, C; BALB/c, B; normal uninfected, N). Following incubation in anti-mouse IgG conjugated to horseradish peroxidase, o-phenylenediamine was added to develop the strips. (b) Plasma samples were pooled from groups of three mice 14 days p.i. with LDV_MUR. Of the four proteins detected by the C58 antibodies, only the one at 48K stained strongly enough to reproduce photographically. (c) Representative strips are shown from an analysis of plasma samples obtained from individual cyclophosphamide-treated C58 and BALB/c mice between 16 and 21 days p.i. (d) Pooled plasma from BALB/c mice 8 months p.i. with LDV_PLA was analysed and the blot was developed using biotin-conjugated anti-mouse IgG followed by avidin-conjugated peroxidase (Sigma); (−) indicates no primary antibody. (e) Following PAGE of 90 μg BALB/c normal mouse plasma, Western blot analysis of pooled immune plasma from BALB/c mice 8 months p.i. (indicated by I) revealed no bands other than those due to background detection of IgG heavy chains (IgGH) which were also seen when blotting with BALB/c normal mouse plasma (N).

Additional experiments were performed in order to determine whether the brain antigens which reacted with LDV-induced IgG were present in other mouse tissues. In Fig. 4 the two Western blot-positive C58 plasma samples from Fig. 3(c) were retested against brain as well as other mouse tissue antigens. This experiment revealed two distinct patterns of antigen reactivity: one in which the brain protein band at 150K was also detected in spleen, liver, kidney, and heart; and a second in which the 70K and 80K brain bands were apparently absent in other mouse tissues. Therefore, some LDV-induced autoantibodies may have tissue-restricted specificity, while others are more broadly reactive.

The Western blot results show that immunological specificity for brain-derived proteins was detected in some plasma samples from 2 to 3 week p.i. C58 mice but in none of the corresponding BALB/c samples. Since all of the mice were antibody-positive by the ELISA test, the blotting technique was apparently unable to resolve all of the detectable antigen reactivity. Nevertheless, the Western blot analyses are significant, not only as an indicator of the specificity
Fig. 4. Western blot analysis of mouse IgG to brain, kidney, liver, spleen and heart. The two brain-positive C58 plasma samples from Fig. 2(c) were tested against C58 brain (lanes 1), liver (lanes 2), kidney (lanes 3), spleen (lanes 4) and heart (lanes 5) as described in Fig. 2. In this experiment, biotinylated anti-mouse IgG and avidin-horseradish peroxidase were used as the detection system, resulting in an artefactual band at 110K due to the presence of an avidin binding site in a protein of this size in some mouse tissues (W. A. Cafruny, unpublished data). Plasma obtained from the C58 mouse at day 18 p.i. with LDV$_{NUR}$ (a) reacted only with the brain proteins of about 70K and 80K, while plasma from the mouse bled at day 21 p.i. (c) reacted with a 150K protein present in all tissues tested. (b) C58 plasma control obtained on day 0 of the experiment was used.

of the anti-brain response, but also by demonstrating that antibodies from BALB/c mice persistently infected for 8 months reacted with a variety of brain-derived proteins. Thus, the nature of these antibodies varies between individual mice and appears to be modulated during the course of viral persistence. The large number of brain-derived proteins detected by IgG from BALB/c plasma 8 months p.i. suggests that this phenomenon has become an immunologically non-specific response to LDV infection at this time. It is not known whether these results have immunopathological implications for the brain lesions which develop in certain LDV-infected mice. At present, it can only be speculated that the anti-brain response might either contribute to or result from the pathological process. In this context it is of interest that recent studies have shown that mice sensitized with spinal cord tissues develop enhanced central nervous system lesions following LDV infection (Stroop & Brinton, 1985), and cyclophosphamide inhibits neither the anti-brain response (this study) nor in certain cases the anti-LDV response (Cafruny et al., 1986a) in C58 mice developing fatal LDV-induced paralysis.

The mechanism by which LDV infection stimulates autoantibodies is not clear, but the data suggest a relationship to the polyclonal B lymphocyte activation associated with LDV infection. Autoantibodies following virus infection have been described previously, and in most experimental systems they are short lived and of low titre (Notkins et al., 1984). Various theories
have been put forward to explain how virus infection may stimulate autoimmunity, including multiple pathways leading to polyclonal activation of lymphocytes (Hirsch & Proffitt, 1975; Rosen et al., 1977; Hutt-Fletcher et al., 1983; Mochizuki et al., 1977; Ahmed & Oldstone, 1984; Butchko et al., 1978; Gibson et al., 1982) and the phenomenon of molecular mimicry (Fujinami et al., 1983; Fujinami & Oldstone, 1985), whereby the virus encodes antigens homologous to host epitopes. The present results indicate that persistent LDV infection stimulates a permanent state of autoimmunity in mice. The mechanism seems most likely to involve the continuing polyclonal activation of immunoglobulins which accompanies LDV infection but which is not found after immunization with inactivated LDV (Cafruny et al., 1986c). Support for this concept is found in studies demonstrating that other polyclonal activators stimulate autoantibodies (Hang et al., 1983; Garzelli et al., 1984), and that clones of autoantibody-producing lymphocytes exist in normal animals (Dresser, 1978; Steele & Cunningham, 1978).

Weiland et al. (1987) have recently described anti-Golgi apparatus autoantibodies resulting from LDV infection. Therefore, our work confirms and extends the autoantibody-producing potential of LDV infection. Further studies are needed to determine whether the autoantigens bound by LDV-induced IgG in the present study are related to antigenic components of the Golgi apparatus. These results are significant in relation to the study of how virus infection causes deregulation of the immune system, and it will be of interest to clarify the mechanisms involved and to determine the pathogenic potential of these antibodies.

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REFERENCES


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