Journal of NeuroVirology (1997) 3, 380–384

http://www.jneurovirol.com

Short Communication

Involvement of CD4$^+$ cells in the protection of C58 mouse against polioencephalomyelitis induced by lactate dehydrogenase-elevating virus

Philippe Monteyne[1,2], Mory Meite[1,3], and Jean-Paul Coutelier[1]

[1]*Unit of Experimental Medicine, International Institute for Cellular and Molecular Pathology, Université Catholique de Louvain, 1200 Bruxelles, Belgium*

Immunosuppression, occurring naturally with aging, or experimentally after cyclophosphamide treatment or irradiation, is required for the development in C58 mice infected with lactate dehydrogenase-elevating virus (LDV) of a severe polioencephalomyelitis that is caused by viral destruction of anterior horn neurons. Here it is shown that depletion of T helper lymphocytes by administration of an anti-CD4 antibody was followed by a progressive paralysis typical of polioencephalomyelitis in C58/J mice inoculated with a neurovirulent strain of LDV. Although it was clear that other cell subsets are also required to assure complete protection of genetically-susceptible mice, our results show that T helper lymphocytes play a major role in the prevention of LDV-induced polioencephalomyelitis. The mechanisms by which these cells confer this protection remain however to be determined.

Keywords: lactate dehydrogenase-elevating virus; T helper lymphocyte; polioencephalomyelitis

Although in most circumstances, lactate-dehydrogenase-elevating virus (LDV) does not induce overt pathology in mice, polioencephalomyelitis may result from infection with this virus when several conditions are met (Martinez *et al*, 1980; Nawrocki *et al*., 1980; Murphy *et al*, 1983). To trigger this paralytic disease, infection, preferably by a neurovirulent strain of LDV (Godeny *et al*, 1993; Faaberg *et al*, 1995; Palmer *et al*, 1995), must occur in mice homozygous for the *Fv-1*^{n} allele (Pease *et al*, 1982), which are permissive for the replication of N-tropic ecotropic MuLV retroviruses. These mice, such as the C58 or AKR animals (Stroop and Brinton, 1983) have to carry several copies of the retrovirus in their genome and retroviral expression in neurons appears to be required for infection and destruction of these cells by LDV (Pease and Murphy, 1980; Contag and Plagemann, 1988, 1989; Inada *et al*, 1993; Anderson *et al*, 1995b). An additional requirement for the development of LDV-induced polioencephalomyelitis is some degree of immunodeficiency that may be achieved by active immunosuppression or by natural depression of immune responses due to an advanced age of the infected animals (Duffey *et al*, 1976). This indicates that at least some immune functions are able to protect mice against the development of this neurologic disease. Despite a protective effect of passive transfer of anti-LDV antibodies (Murphy *et al*, 1983; Harty *et al*, 1987; Harty and Plagemann, 1990; Anderson *et al*, 1995a), the possible role of naturally produced antiviral antibodies in the prevention of polioencephalomyelitis development is not definitely demonstrated (Cafruny *et al*, 1986). In contrast, T lymphocytes are clearly required for protection against this neurological disease (Bentley and Morris, 1982). The protective subpopulations of T cells have been analysed with Lyt-1 and Lyt-2 (currently CD8) markers. The CD8$^+$ cells are clearly involved (Bentley and Morris, 1982; Bentley *et al*, 1983). However, so far, a possible role for CD4$^+$ T lymphocytes has not been investigated. The aim of the present work was therefore to determine whether these T helper lymphocytes were required to protect genetically-susceptible mice against the

Correspondence: J-P Coutelier, Unité de médecine expérimentale, UCL MEXP 7430, Avenue Hippocrate 74, 1200 Bruxelles, Belgium. Tel: 32 2 764 7437; fax:32 2 764 7430.
Present addresses: [2]Unité des virus lents, Institut Pasteur, 75724 Paris Cedex 15, France. [3]Service d'hématologie et d'immunologie, CHU de Yopougon, 21 BP 632 Abidjan 21, Ivory Coast
Received 2 January 1997; revised 21 April 1997; accepted 4 June 1997

development of LDV-induced polioencephalomyelitis.

Female C58/J mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) and maintained in microisolators with sterile food and water. Polioencephalomyelitis was induced in these animals by immunosuppression and by intraperitoneal injection of LDVc (kindly given by EK Godeny, Martinez *et al*, 1980). This virus was amplified once in C58/J mice and the equivalent of 2 μl plasma from 1 day-infected mice, diluted in 500 μl saline was used for infection. Immunosuppression was obtained either with 5 mg cyclophosphamide (Cycloblastine^{R}, Farmitalia Carlo Erba), or by 600 rads whole body irradiation. The intensity of the disease was estimated with an arbitrary score as follows: 1=paresis of one or two legs; 2=paresis of more than two legs; 3=flaccid paralysis of more than two legs; 4=total flaccid paralysis; 5=death. Regardless of the type of immunosuppression, a progressive and severe paralysis developed about 2 weeks after infection, leading to death in about 50% of the animals (shown in Figure 1a, after irradiation).

To determine whether CD4^{+} T lymphocytes are part of the immune mechanisms that prevent the development of polioencephalomyelitis in normal animals, depletion of these cells was achieved by *in vivo* injection of the GK1.5 anti-CD4 monoclonal antibody (made available by FW Fitch, and obtained through the courtesy of HR MacDonald). It has been shown previously that this antibody was able to deplete T helper lymphocytes *in vivo* (Coulie *et al*, 1985; Coutelier *et al*, 1990). In C58/J mice, nearly complete disappearance of CD4^{+} cells was obtained in preliminary experiments after injection of GK1.5 doses ranging from 0.25 mg to 1 mg. Indeed, as measured by cell labelling as described previously (Coutelier *et al*, 1994), the fraction of CD4^{+} cells in total spleen cells dropped from 25.5% in control untreated mice to about 0.2% after anti-CD4 treatment. Thus, 5–6 month-old C58/J mice were treated with 1 mg GK1.5 antibody 2 days before administration of LDVc, followed by 0.5 mg of the same antibody 5 and 12 days after infection. As shown in Figure 1b for a typical experiment, all the mice that received anti-CD4 treatment in addition to LDVc infection developed a gradual paralysis, whereas animals that were only infected were not affected. This result demonstrates that T helper lymphocytes are required to prevent this neurological disease in genetically-susceptible animals infected with LDVc. Interestingly, polioencephalomyelitis induced by anti-CD4 treatment was not always as severe as the disease following irradiation, and many animals, although affected by the paralysis, did survive (Figure 1). Moreover, in younger mice (2–3 month-old), whereas the po-

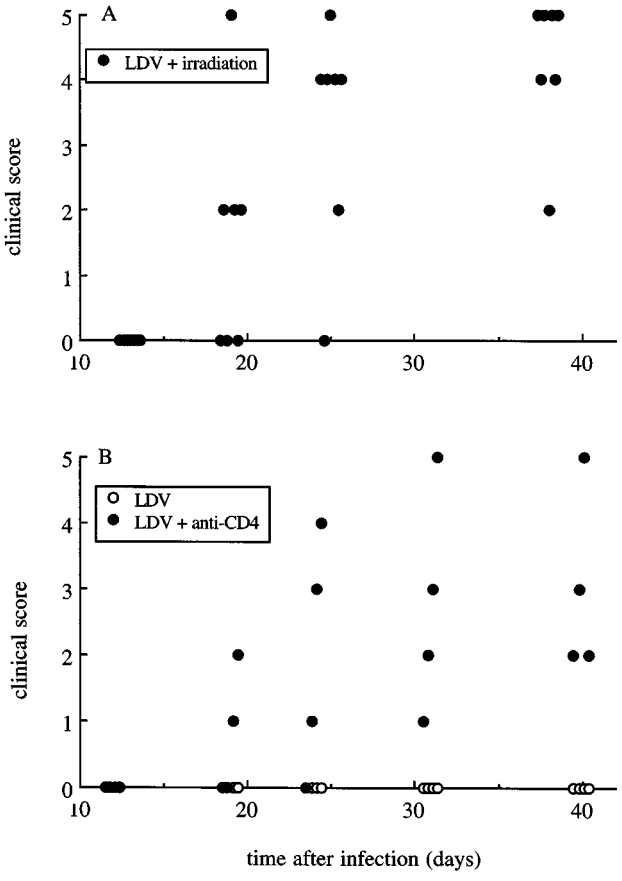

Figure 1 Development of polioencephalomyelitis in immunosuppressed C58/J mice. Five to six month-old female C58/J were infected with LDVc (**a**) 2 days after immunosuppression by 600 rads whole body irradiation; (**b**) without (open symbols) or with (closed symbols) treatment with anti-CD4 GK1.5 antibody, as indicated in the text. Development of polioencephalomyelitis was determined by clinical score of the paralysis at different times after infection.

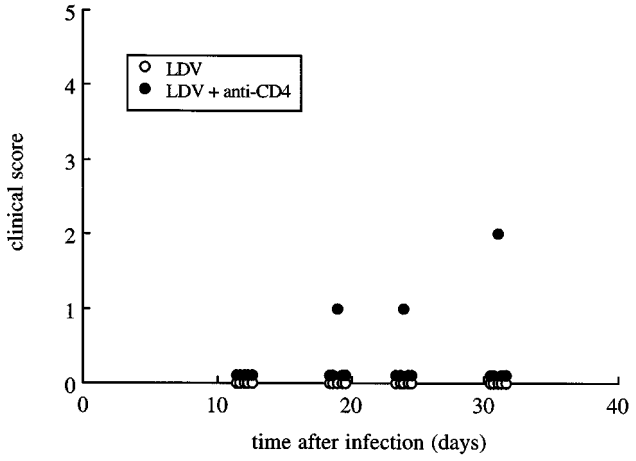

Figure 2 Induction of polioencephalomyelitis in young C58/J mice by anti-CD4 treatment. Five 2–3 month-old C58/J mice were infected with LDVc without (open symbols) or with (closed symbols) treatment with anti-CD4 GK1.5 antibody. Development of polioencephalomyelitis was determined by clinical score of the paralysis at different times after infection.

lioencephalomyelitis was occasionally observed, it was only in a few animals (Figure 2). These data which fit well with previous observations in young mice treated with cyclophosphamide (Anderson *et al*, 1995a), suggest that in addition to $CD4^+$ cells, other cells that disappear with the age of the animals are involved in the protection against LDV-triggered paralysis. That these cells may be $CD8^+$ lymphocytes has been indicated by experiments reported by others (Bentley and Morris, 1982; Bentley *et al*, 1983) and was confirmed by treatment of infected mice with an anti-CD8 antibody (53/6.72, rat IgG2a, from ATCC, Ledbetter and Herzenberg, 1979). In preliminary experiments, inoculation of 1 ml ascitic fluid containing this antibody was found to depress the $CD8^+$ fraction in spleen cells from 11–18% in control C58/J mice to 0.5–2% 3 days after antibody treatment. Combined data from two independent experiments indicated that anti-CD8 treatment was followed by the development of a moderate neurological disease in about half of the infected animals (three out of a total of eight mice that received anti-CD8 antibody). Such a modest effect of anti-CD8 antibody treatment, compared with the more dramatic effect of anti-CD4, might be due to the incomplete elimination of $CD8^+$ cells after antibody inoculation.

Our results clearly indicate that, in addition to $CD8^+$ cells, $CD4^+$ lymphocytes play a major role in the protection of susceptible mice against LDVc-induced polioencephalomyelitis. Interestingly, a collaboration between these two cell subpopulations is also required for successful defence against other viruses, especially in the central nervous system (Williamson and Stohlman, 1990). The mechanisms by which $CD4^+$ T cells prevent the neurological disease triggered by LDV are still unknown. Preliminary results of cytokine analysis in the central nervous system of infected animals indicate that in absence of immunosuppression gamma-interferon is produced. This suggests that protective $CD4^+$ lymphocytes may be of the Th1 phenotype, which would be in agreement with the characteristics of the general immune responses usually observed in mice infected with LDV (Coutelier and Van Snick, 1985; Coutelier *et al*, 1986, 1995; Li *et al*, 1990; Monteyne *et al*, 1993). However, whether the protective ability of a Th1 response would be better than that of a Th2 response is yet to be analysed. It remains also to be demonstrated whether the ability of lymphocytes to prevent polioencephalomyelitis results from some polyclonal activation resulting in cytokine production or from an effector function linked to their antigenic specificity, against some viral antigen. For instance, the ability of $CD4^+$ T lymphocytes of the Th1 phenotype to lyse target cells infected with some viruses has been demonstrated (Heemskerk *et al*, 1995). It has also been shown that the presence of functional $CD4^+$ T cells is required for the production of anti-LDV antibodies by B lymphocytes (Coutelier *et al*, 1986; Onyekaba *et al*, 1989). Although we were not able to detect by ELISA, as described previously (Coutelier *et al*, 1986) significant $CD4^+$ cell-dependent production of anti-LDV antibodies in immunocompetent C58 mice during the first three weeks of infection (data not shown) and whereas it has been reported by indirect fluorescent antibody assay that some C58 animals paralysed after LDV infection did develop consistent antiviral antibody responses (Anderson *et al*, 1995a), it cannot be ruled out that the protective effect of $CD4^+$ T lymphocytes involves, at least partly, help to B lymphocytes to produce neutralising antibodies.

Acknowledgements

The authors are indebted to Drs M Brahic and PL Masson for critical reading of this manuscript and to T Briet, M-D Gonzales, N Havaux and J Van Broeck for expert technical assistance. Drs EK Godeny, P Coulie, FW Fitch and HR MacDonald are gratefully acknowledged for gift of reagents.

This work was supported by the Fonds National de la Recherche Scientifique (FNRS), Fonds de la Recherche Scientifique Médicale (FRSM), Loterie Nationale and the State-Prime Minister's Office - SSTC (interuniversity attraction poles, grant no 44) and the French Community (concerted actions, grant no 88/93-122), Belgium. J-PC is a senior research associate with the FNRS.

References

Anderson GW, Even C, Rowland RRR, Palmer GA, Harty JT, Plagemann PGW (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 GW, Palmer GA, Rowland RRR, Even C, Plagemann PGW (1995b). Infection of central nervous system cells by ecotropic murine leukemia virus in C58 and AKR mice and in inutero-infected CE/J mice predisposes mice to paralytic infection by lactate dehydrogenase-elevating virus. *J Virol* **69:** 308–319.

Bentley DM, Morris RE (1982). T cell subsets required for protection against age-dependent polioencephalomyelitis. *J Immunol* **128:** 530–534.

Bentley DM, Watson SR, Morris RE (1983). Age-related loss of Lyt-1,2 cells in C58 mice results in susceptibility to lactic dehydrogenase virus-induced polioencephalomyelitis. *Inf Immun* **41:** 1389–1390.

Cafruny WA, Strancke CR, Kowalchyk K, Plagemann PGW (1986). Replication of lactate dehydrogenase-elevating virus in C58 mice and quantification of antiviral antibodies and of tissue virus levels as a function of development of paralytic disease. *J Gen Virol* **67:** 27–37.

Contag CH, Plagemann PGW (1988). Susceptibility of C58 mice to paralytic disease induced by lactate dehydrogenase-elevating virus correlates with increased expression of endogenous retrovirus in motor neurons. *Microb Pathog* **5:** 287–296.

Contag CH, Plagemann PGW (1989). Age-dependent poliomyelitis of mice: expression of endogenous retrovirus correlates with cytocidal replication of lactate dehydrogenase-elevating virus in motor neurons. *J Virol* **63:** 4362–4369.

Coulie PG, Coutelier JP, Uyttenhove C, Lambotte P, Van Snick J (1985). In vivo suppression of T-dependent antibody responses by treatment with a monoclonal anti-L3T4 antibody. *Eur J Immunol* **15:** 638–640.

Coutelier JP, Coulie PG, Wauters P, Heremans H, van der Logt JTM (1990). In vivo polyclonal B-lymphocyte activation elicited by murine viruses. *J Virol* **64:** 5383–5388.

Coutelier JP, Johnston SJ, El Azami El Idrissi M, Pfau CJ (1994). Involvement of CD4+ cells in lymphocytic choriomeningitis virus-induced autoimmune anaemia and hypergammaglobulinaemia. *J Autoimmunity* **7:** 589–599.

Coutelier JP, Van Broeck J, Wolf SF (1995). Interleukin-12 gene expression after viral infection in the mouse. *J Virol* **69:** 1955–1958.

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

Coutelier JP, Van Snick J (1985). Isotypically restricted activation of B lymphocytes by lactic dehydrogenase virus. *Eur J Immunol* **15:** 250–255.

Dialynas DP, Wilde DB, Marrack P, Pierres A, Wall KA, Havran W, Otten G, Loken MR, Pierres M, Kappler J, Fitch FW (1983). Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II antigen-reactivity. *Immunological Rev* **74:** 29–56.

Duffey PS, Martinez D, Abrams GD, Murphy WH (1976). Pathogenic mechanisms in immune polioencephalomyelitis: induction of disease in immunosuppressed mice. *J Immunol* **116:** 475–481.

Faaberg KS, Palmer GA, Even C, Anderson GW, Plagemann PGW (1995). Differential glycosylation of the ectodomain of the primary envelope glycoprotein of two strains of lactate dehydrogenase-elevating virus that differ in neuropathogenicity. *Virus Res* **39:** 331–340.

Godeny EK, Chen L, Kumar SN, Methven SL, Koonin EV, Brinton MA (1993). Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV). *Virology* **194:** 585–596.

Harty JT, Chan SPK, Contag CH, Plagemann PGW (1987). Protection of C58 mice from lactate dehydrogenase-elevating virus-induced motor neuron disease by non-neutralizing antiviral antibodies without interference with virus replication. *J Neuroimmunol* **15:** 195–206.

Harty JT, Plagemann PGW (1990). Monoclonal antibody protection from age-dependent poliomyelitis: implications regarding the pathogenesis of lactate dehydrogenase-elevating virus. *J Virol* **64:** 6257–6262.

Heemskerk MHM, Schoemaker HM, Spaan WJM, Boog CJP (1995). Predominance of MHC class II-restricted CD4+ cytotoxic T cells against mouse hepatitis virus A59. *Immunology* **84:** 521–527.

Inada T, Kikuchi H, Yamazaki S (1993). Comparison of the ability of lactate dehydrogenase-elevating virus and its virion RNA to infect murine leukemia virus-infected or -uninfected cell lines. *J Virol* **67:** 5698–5703.

Ledbetter JA, Herzenberg LA (1979). Xeno-geneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol Rev* **47:** 63–90.

Li X, Hu B, Harty J, Even C, Plagemann PGW (1990). Polyclonal B cell activation of IgG2a and IgG2b production by infection of mice with lactate dehydrogenase-elevating virus is partly dependent on CD4+ lymphocytes. *Viral Immunology* **3:** 273–288.

Martinez D, Brinton MA, Tachovsky TG, Phelps AH (1980). Identification of lactate dehydrogenase-elevating virus as the etiological agent of genetically restricted, age-dependent polioencephalomyelitis of mice. *Infect Immun* **27:** 979–987.

Monteyne P, Van Broeck J, Van Snick J, Coutelier JP. (1993). Inhibition by lactate dehydrogenase-elevating virus of in vivo interleukin 4 production during immunization with keyhole limpet haemocyanin. *Cytokine* **5:** 394–397.

Murphy WH, Nawrocki JF, Pease LR (1983). Age-dependent paralytic viral infection in C58 mice: possible implications in human neurologic disease. *Prog Brain Res* **59:** 291–303.

Nawrocki JF, Pease LR, Murphy WH (1980). Etiologic role of lactic dehydrogenase virus infection in an age-dependent neuroparalytic disease in C58 mice. *Virology* **103:** 259–264.

Onyekaba CO, Harty JT, Even C, Hu Bg, Plagemann PGW (1989). Persistent infection of mice by lactate dehydrogenase-elevating virus: effects of immunosuppression on virus replication and antiviral immune responses. *Virus Research* **14:** 297–316.

Palmer GA, Kuo L, Chen Z, Faaberg KS, Plagemann PG (1995). Sequence of the genome of lactate dehydrogenase-elevating virus: heterogenicity between strains P and C. *Virology* **209:** 637–642.

Pease LR, Abrams GD, Murphy WH (1982). FV-1 restriction of age-dependent paralytic lactic dehydrogenase virus infection. *Virology* **117:** 29–37.

Pease LR, Murphy WH (1980). Co-infection by lactic dehydrogenase virus and C-type retrovirus elicits neurological disease. *Nature (London)* **286:** 398–400.

Stroop WG, Brinton MA (1983). Mouse strain-specific central nervous system lesions associated with lactate dehydrogenase-elevating virus infection. *Lab Invest* **49:** 334–345.

Williamson JSP, Stohlman SA (1990). Effective clearance of mouse hepatitis virus from the central nervous system requires both CD4+ and CD8+ T cells. *J Virol* **64:** 4589–4592.