The role of CD8\(^+\) T cells in the acute and chronic phases of Theiler's murine encephalomyelitis virus-induced disease in mice

P. Borrow,\(^\dagger\) P. Tonks, C. J. R. Welsh\(^\ddagger\) and A. A. Nash\(^*\)

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1Q P, U.K.

The technique of \textit{in vivo} depletion with T cell subset-specific monoclonal antibodies was used to study the involvement of CD8\(^+\) T cells in protection/pathogenesis during the acute and chronic demyelinating phases of Theiler's murine encephalomyelitis virus (TMEV)-induced disease. Mice rendered CD8-deficient prior to infection with TMEV were less efficient at clearing virus from the central nervous system compared to intact animals and also suffered demyelinating disease of earlier onset and increased severity. This indicates that CD8\(^+\) cells have a protective role in virus clearance at early times post-infection, and may also be involved in downregulating the severity of the chronic demyelinating disease. How CD8\(^+\) T cells function to produce these effects is discussed.

TO isolates of the picornavirus Theiler's murine encephalomyelitis virus (TMEV) induce a biphasic disease of the central nervous system (CNS) in susceptible mouse strains (Lipton, 1975, 1980). The acute disease occurs 2 to 20 days after intracranial infection and resembles poliomyelitis. Virus replicates in the grey matter (predominantly within motor neurons) of the brain and spinal cord, giving rise to a flaccid paralysis. Surviving animals go on to develop a chronic CNS disease with inflammatory demyelinating lesions confined to the white matter (Aubert \textit{et al.}, 1987).

Although the acute disease is clearly a result of direct viral damage, the pathogenesis of the demyelinating disease is less well understood. It too may be virus-mediated, as the virus persists in the white matter (Brahic \textit{et al.}, 1981) and it has been shown that oligodendrocytes are the principal cell type infected (Rodriguez \textit{et al.}, 1983). However, there is also evidence to suggest that demyelination is in part immunemediated. The lesions have a distribution and appearance similar to those in experimental autoimmune encephalomyelitis, being associated with inflammatory cell infiltrates (Dal Canto & Lipton, 1975; Blakemore \textit{et al.}, 1988). Their incidence and severity is reduced by immunosuppressive therapies (Lipton & Dal Canto, 1976, 1977; Roos \textit{et al.}, 1982) including treatment with anti-Ia (Rodriguez \textit{et al.}, 1986a; Friedmann \textit{et al.}, 1987) and anti-CD4 antibodies (Welsh \textit{et al.}, 1987) suggesting that major histocompatibility complex (MHC) class II-restricted CD4\(^+\) T cells are involved in pathogenesis.

Immunogenetic experiments indicate that at least one of the genes that determine disease susceptibility/resistance in inbred mouse strains is MHC-associated (Lipton & Melvold, 1984; Rodriguez & David, 1985; Clatch \textit{et al.}, 1985, 1987a, b), although the locus involved has been mapped to the 5' end of the H-2D gene, implying a MHC-class I-restricted effect (Rodriguez \textit{et al.}, 1986b). This observation suggests that CD8\(^+\) T cells may also participate in immunity to, or the immunopathology of, this virus infection. To study the role of CD8\(^+\) T cells in the immune response to TMEV, we used the technique of \textit{in vivo} depletion of T cells with mouse CD8-specific monoclonal antibodies (Cobbold \textit{et al.}, 1984). In thymectomized mice, CD8\(^+\) T cells remain depleted for the lifetime of the animal whereas the level of CD4\(^+\) T cells remains unaffected (Nash \textit{et al.}, 1987). By measuring the clearance of virus from the brain and spinal cord and assessing clinical signs of demyelination, a role for CD8\(^+\) T cells in the immune response and disease process induced by this virus could be deduced.

In two parallel series of experiments in female SJL mice (obtained from Olac Laboratories, Bicester, U.K.) and CBA mice (from the Department of Pathology Animal Unit, Cambridge University, U.K.), groups of intact and CD8\(^+\) cell-depleted animals were infected intracranially (i.c.) with the BeAn strain of TMEV, and their mortality and clinical signs of disease were monitored over a 12 week period.
The BeAn 8386 strain of TMEV was a gift from Dr H. L. Lipton (Department of Neurology, University of Colorado Health Sciences Centre, Denver, Co., U.S.A.); stocks were grown in BHK 21 cells and stored at -70°C prior to use. Mice were infected (day 0 of the experiment) by anaesthetizing with ether and injecting in the right cerebral hemisphere with 10^4 p.f.u. of BeAn in 20 µl of Glasgow MEM. Uninfected control animals were injected with 20 µl of sterile PBS. Mice to be T cell-depleted were thymectomized prior to infection, by standard surgical procedures under hypnorm-valium anaesthesia; undepleted control animals underwent a sham thymectomy operation.

Thymectomized mice were rendered CD8⁺ cell-deficient by two intravenous (i.v.) injections, on days -1 and +2 relative to the time of infection (day 0) of 0.1 ml of the rat IgG2b monoclonal antibody YTS 169.4 against murine CD8 (Cobbold et al., 1984). The antibody was partially purified from ascites fluid by precipitation with 50% (NH₄)₂SO₄, dialysed against PBS, and stored in aliquots at -20°C at a concentration of 10 mg protein/ml. This depletion procedure has been shown to result in a permanent elimination of 98% of subset-specific T cells (Cobbold et al., 1984); the efficiency of depletion achieved here was checked by staining peripheral blood smears in samples from mice in each experiment for their CD8⁺ cell content, using the staining protocol described by Cobbold et al. (1986), and was found to be >95%. The level of CD4⁺ T cells in the CD8⁺ cell-depleted mice was unaffected.

The BeAn 8386 strain of TMEV used for infection was tissue culture-adapted, and produced only mild signs of the early polio-like disease phase in intact SJL and CBA mice: only one of 20 (5%) SJL and four of 25 (16%) CBA mice developed complete paralysis of one or both hind limbs. CD8⁺ cell-depleted animals exhibited slightly more severe acute-phase signs than did the undepleted controls, but there was no difference in mortality; the early disease very rarely proved fatal in either group.

Clinical signs of the chronic, demyelinating disease were scored twice weekly (without prior knowledge of the experimental protocol) on a scale from 0 to 6, where 0 represents a healthy and 6 a dead animal and intermediate numbers a gradual increase in severity of disease signs including ruffling, hunching, unsteady gait, weak grasp response, paralysis and diarrhoea (assessed as previously described; Welsh et al., 1987). Histological analysis showed that there were always signs of demyelination in the CNS of both SJL and CBA mice with clinical scores of 3 or more (i.e. exhibiting moderate to severe clinical signs of the chronic disease). In Fig. 1, the percentage of intact and CD8⁺ cell-depleted mice with a clinical score of 3 or more over the 12 week period following infection with BeAn is shown to illustrate disease onset. SJL mice are more susceptible than are CBA mice, and the onset of disease is more rapid in this strain. For both mouse strains, however, it was evident that the CD8⁺ cell-depleted animals showed a more rapid onset of disease than did intact mice, and a higher percentage of depleted animals were more severely affected throughout. This was seen more clearly in CBA mice, where the mean week of onset of clinical symptoms of demyelination in the CD8⁺ cell-depleted animals was 7.9 ± 3.6 compared to 11.8 ± 5.3 in the intact group. However, the difference in the percentage of SJL mice affected in the two groups was also statistically signifi-
When intact and CD8\(^+\) cell-depleted mice were sampled 4 to 12 weeks post-infection (p.i.) for histological analysis of the CNS, as inferred from the clinical scores more CD8\(^+\) cell-depleted animals of each strain showed histological evidence of demyelination than did intact mice. However, there was no obvious histological difference in the type of lesions in intact and CD8\(^+\) cell-depleted animals (data not shown).

From these results it can be concluded that mice depleted of CD8\(^+\) T cells prior to infection with TMEV suffer from a more severe demyelinating disease of rapid onset than do undepleted animals. Thymectomy alone does not affect T cell responses in adult mice (Nash et al., 1987). This would suggest that CD8\(^+\) cells normally have a protective role in the demyelinating disease phase and/or that as a result of their absence during the early disease phase the subsequent late disease is more severe.

To address the question of whether CD8\(^+\) cells play an important protective role during chronic infection, a large group of SJL mice, half of which had been thymectomized, were infected with BeAn, then 4 weeks p.i., CD8\(^+\) T cells were depleted from the thymectomized animals by giving two i.v. injections of 0.1 ml of monoclonal antibody YTS 169.4 on days 28 and 31 p.i. The clinical signs of demyelinating disease that subsequently developed in intact and depleted animals were then compared. There was no significant difference in the mean clinical score of the two groups of animals from 4 to 12 weeks p.i.; there was also no difference in CNS virus titres in animals sampled at weekly intervals from the two groups (data not shown).

This would suggest that CD8\(^+\) T cells are not a major component in either protection or immunopathogenesis in the chronic disease phase in TMEV-infected mice. However, this result must be interpreted with caution in that although it was shown that the majority of CD8\(^+\) T cells were depleted from the periphery of these animals by monoclonal antibody treatment, the extent of depletion of activated T cells achieved within the CNS is not known. Even following damage to the blood–brain barrier, cells within the CNS may be sequestered to some extent from the monoclonal antibody. Alternatively, activated cells may be resistant to the in vivo depleting effects of T cell subset-specific monoclonal antibodies (Sriram et al., 1988). At day 28 p.i. when anti-CD8 treatment was begun, virus-specific CD8\(^+\) T cells which had been recently activated in response to viral infection might thus be resistant to the effects of the monoclonal antibody, despite the widespread depletion of ‘resting’ CD8\(^+\) cells.

If CD8\(^+\) T cells do not have an important protective role at late times p.i., the observation that mice depleted of CD8\(^+\) cells suffer more severely from the chronic demyelinating disease could be due to these cells being involved in virus clearance in the first month p.i. If virus clearance was less effective in CD8\(^+\) cell-depleted animals, and the titre of virus persisting in the CNS at late times p.i. was higher, this could account for the late disease in these animals being more severe. CNS virus titres in intact mice and mice depleted of CD8\(^+\) cells prior to infection were therefore compared.

Groups of intact and CD8\(^+\) cell-depleted SJL and CBA mice were infected i.c. with BeAn, and at various times p.i. mice were sampled at random and virus titres in the brain and spinal cord determined by plaque assay on BHK 21 cells using the method of Rueckert & Pallansch (1981). The brain virus titres are shown in Fig. 2(a) and (b) (SJL mice) and 2 (c) and (d) (CBA mice). Once again, similar conclusions can be drawn from the findings in both mouse strains.

CNS virus titres rose over the first 1 to 2 weeks p.i., reaching similar maximum levels (approximately 10\(^6\) p.f.u.) in both intact and CD8\(^+\) cell-deficient animals. Virus clearance then began, but whereas the majority of intact mice (three of three SJL and five of six CBA mice) cleared the virus to levels below those detectable by the plaque assay (<10 p.f.u./g tissue) by 4 weeks p.i., clearance was delayed to 5 weeks in the CD8\(^+\) cell-deficient animals. At late times p.i., virus was detected in the CNS of more CD8\(^+\) cell-depleted animals (in the brains of nine of 18 SJL mice assayed 4 to 12 weeks p.i.) than in undepleted mice (two of 18 SJL brains assayed 4 to 12 weeks p.i.). This formed part of a general correlation between CNS virus titres and the severity of clinical signs of demyelinating disease in individual animals. It is important to note that the effect of thymectomy on the course of virus replication in the CNS and on the resulting disease is similar to that observed in euthymic mice (Welsh et al., 1987).

The results presented so far indicate that CD8\(^+\) T cells do contribute towards protection in TMEV-infected mice, a conclusion supported by unpublished results discussed by Clatch et al. (1987a) who also suggest that disease onset is exacerbated in CD8\(^+\) cell-depleted mice. In contrast to this, Rodriguez & Sriram (1988) presented experiments in which they depleted CD8\(^+\) cells from DA-infected SJL mice either immediately prior to infection or on days 15 to 17 p.i., and showed that the demyelinating disease in these animals was less severe, suggesting that CD8\(^+\) cells have an immunopathological role in TMEV-induced disease. They failed to observe higher CNS virus titres in CD8\(^+\) cell-depleted mice, even when depletion was carried out at the time of infection. This could be explained by differences in the strain of TMEV used, or because the depleted animals had not
been thymectomized, and so repopulating CD8+ cells were able to carry out the normal functions of this T cell subset. Their observation that CD8+ T cells have an immunopathological role in the chronic disease phase is based largely on histological differences observed at later times p.i. (when peripheral CD8+ T cells had reached 40 to 50% of normal levels), but the actual differences involved were not very large.

If, as our data suggest, CD8+ cells do have a protective role in TMEV-infected mice, how do they act to bring about this effect?

Neutralizing antibody production is known to be of vital importance in virus clearance in TMEV-infected mice. We have shown previously that mice depleted of CD4+ T cells prior to infection with TMEV are unable to generate an antiviral antibody response and die of acute encephalitis approximately 4 weeks p.i. (Welsh et al., 1987). Although T_H for antibody production are predominantly CD4+ cells, it was possible that CD8+ cells might also be involved in the upregulation of antibody production in TMEV-infected mice (e.g. if a CD8+ cell subset equivalent to the CD4+ T_H2 subset exists).

However, when serum antiviral antibody levels were measured by ELISA and neutralizing antibody titres determined in intact and CD8+ cell-deficient mice, no difference was observed in the quantity, class or quality of serum antibody responses developed with time p.i. in the absence of CD8+ T cells (not shown).

The classical protective role for CD8+ T cells in many viral infections is as cytotoxic T lymphocytes (CTLs). It has recently been shown that H-2-restricted TMEV-specific CTL activity mediated by CD8+ T cells can be demonstrated in lymphocytes isolated from TMEV-infected mice (Lindsley et al., 1991; Rossi et al., 1991). Virus-specific CTL activity is present at 7 days p.i., and has been demonstrated as late as day 226 p.i. in the CNS of SJL mice. Thus in TMEV-infected mice CD8+ T cells acting as CTLs may work in concert with antiviral antibody both to clear the initial burst of virus replication and to control virus persisting in the CNS at late times p.i.

In summary, the results presented here clearly demonstrate that CD8+ T cells contribute to protection in TMEV-infected mice. This may be due to CD8+ T cells...
with CTL activity clearing virus from infected cells and reducing CNS virus titres. Much work remains to be done, both to clarify further the mechanisms involved in virus clearance in TMEV-infected mice, and to cast more light on factors that influence strain-specific differences in susceptibility to demyelinating disease produced following infection of mice with TMEV.

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References


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