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Dexamethasone regulation of interleukin-1-receptors in the hippocampus of Theiler's virus-infected mice: effects on virus-mediated demyelination

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Abstract

Intracerebral (i.c.) inoculation of susceptible strains of mice with Theiler's murine encephalomyelitis virus (TMEV) results in immune-mediated demyelinating disease. Interleukin-1 receptors are expressed in the brain of mice, in particular in the hippocampus, and have been implicated in neuroimmunoendocrine interactions. In the present study we investigated the regulation of interleukin-1 receptors in the hippocampus of a susceptible (SJL/J) and a resistant (BALB/c) strain of mice infected with TMEV, at different time intervals of the disease. Our results show that interleukin-1 receptors in the hippocampus were decreased in TMEV-infected mice at early times post-infection (10 and 14 days p.i.). The reduction in interleukin-1 receptors only occurred in the susceptible strain of mice (SJL/J), whereas interleukin-1 binding in the hippocampus of TMEV-infected resistant mice (BALB/c) showed values similar to those in control animals. The TMEV-induced down-regulation of interleukin-1 receptors was secondary to a marked decrease in the affinity of the receptor (control: $K_d = 10.5$ pM; TMEV: $K_d = 1.30$ pM) accompanied by a decrease in receptor number (control: $B_{max} = 2.189$ fmol/mg protein; TMEV: $B_{max} = 0.84$ fmol/mg protein). We also investigated the effects of glucocorticoid treatment on the regulation of hippocampal interleukin-1 receptors of TMEV-infected mice. Dexamethasone treatment in the early phase (500 μg/kg or 1 mg/kg during days 5–10 p.i.) of the disease significantly reversed the deficits in hippocampal interleukin-1 receptors observed at 10 days p.i. in SJL/J mice, and suppressed neurological signs of demyelination. These results suggest that: (i) the reduction of interleukin-1 receptors may be a consequence, at least in part, of local production of interleukin-1 at early times during TMEV infection; (ii) interleukin-1 seems to be a critical factor for the susceptibility to TMEV-induced demyelination and (iii) the protective effect of dexamethasone appears to be related to its ability to reverse the reduction in interleukin-1 receptors during the early disease. These results suggest that interleukin-1 is a pivotal mediator in TMEV-induced demyelination.

Keywords: Interleukin-1 receptor; Hippocampus; Dexamethasone; Theiler's murine encephalomyelitis virus

1. Introduction

Susceptible mice experimentally infected with certain strains of Theiler's murine encephalomyelitis virus (TMEV), develop a biphasic neurological disease, characterised by chronic inflammatory demyelination of the central nervous system (CNS) (Lipton, 1975). Theiler's original (TO) strains of TMEV virus (strains DA and BeAn) injected intracerebrally (i.c.) to SJL/J mice induce an acute phase that develops from the 1st to the 3rd week post-infection (p.i.) and resembles acute poliomyelitis. Animals that survive this acute phase, and animals infected with TO viruses attenuated by culture passages, develop the second phase of the disease characterised by chronic demyelination with viral persistence in the spinal cord. This late phase of Theiler's virus encephalomyelitis is used as a model of multiple sclerosis. While the etiology of multiple sclerosis is still unknown, it has been speculated that the disease can be triggered by a virus and that its development is mediated by immune mechanisms (Waksman and Reingold, 1986; Tsunoda and Fujinami, 1996; Monteney et al., 1997). Most neuropathological studies have been done with brains of multiple sclerosis patients who died after long-term disease. However, controversy arises when one tries to study the initial pathological events and possible early triggers of this demyelinating disease. TMEV infection leads to an immune-mediated inflammatory reaction mediated by a virus-specific T-cell response and non-specific macrophage activation (Lipton and Dal Canto, 1976; Clatch et al., 1986, 1987; Lindsley

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and Rodriguez, 1989). These macrophages and some myelinotoxic cytokines secreted during the inflammatory response destroy myelin sheets (Lindsley and Rodriguez, 1989; Levy et al., 1992; Pena Rossi et al., 1997).

The interleukin-1 family of peptides (interleukin-1α, interleukin-1β interleukin-1 receptor antagonist) induces centrally host defense responses to infectious pathogens (Dinarello, 1996). Brain interleukin-1 has also been implicated in acute and chronic neurodegenerative diseases (Rothwell, 1991). Interleukin-1 is one of the pro-inflammatory cytokines related to TMEV infection of SJL/J mice (Rubio and Torres, 1991), and it is increased in astrocyte cultures infected with this virus (Rubio and Capa, 1993). A contributory role of cytokines in the induction of demyelinating disease is suggested by results of several studies. Interferon-gamma blockade by monoclonal antibodies increases disease severity and mortality of TMEV encephalomyelitis (Kohanawa et al., 1993). Exogenous administration of interleukin-1 and interleukin-2 increases disease severity in Coxsackie virus B3 infection (Hubber et al., 1994), and injections of antibody to soluble interleukin-1 receptors suppress experimental allergic encephalomyelitis, while interleukin-1α administration worsens the condition (Jacobs et al., 1991). Furthermore, interleukin-1 receptor antagonist has been shown to suppress experimental allergic encephalomyelitis in rats (Badovinac et al., 1998).

Two distinct interleukin-1 receptors have been described, the type 1 receptor is found mainly on T-cells and fibroblasts, while the type 2 receptor is found mainly on B-cells and neutrophils (Sims et al., 1994). While type I interleukin-1 receptor appears to mediate all biological effects of interleukin-1, type II interleukin-1 receptor is important as decoy which modifies interleukin-1 actions. Interleukin-1 receptors have been identified in various murine brain structures as well as in the anterior pituitary (Ban et al., 1991; Takao et al., 1993; Betancur et al., 1994). A high-affinity interleukin-1 receptor, similar to type I receptors (80 kDa), described on T-lymphocytes (80 kDa), is mainly expressed in the hippocampus, in the cortex, the choroid plexus and the meninges. Furthermore, the localisation of [^{125}I]interleukin-1α binding sites corresponds exactly to the anatomical localisation of type I interleukin-1 receptor mRNA, identified mainly in the dentate gyrus of the hippocampal formation (Cunningham et al., 1992). Using reverse transcription–polymerase chain reaction (RT–PCR), the presence of the two types of receptor transcripts was demonstrated in the mouse brain (Parnet et al., 1994)

Interleukin-1 receptor expression can be negatively regulated by interleukin-1 in several kinds of cells such as monocytes or fibroblasts (Matsushima et al., 1986; Mizel et al., 1987). Interestingly, a strong decrease in interleukin-1 receptors density in hippocampi has been observed following peripheral (Takao et al., 1993) or intravenous lipopolysaccharide injections (Haour et al., 1990). Experiments showing that, after systemic LPS interleukin-1 mRNA (Ban et al., 1992; Layé et al., 1994) is induced, suggest that i.c. interleukin-1 synthesis could account for the receptor number regulation by LPS.

The hypothalamus–pituitary–adrenocortical axis plays a critical role in the interactions between the immune and neuroendocrine systems. Several cytokines, in particular interleukin-1, are able to stimulate the activity of the hypothalamus–pituitary–adrenocortical axis at different levels resulting in increased serum levels of adrenocorticotropin hormone and glucocorticoids (Berkenbosch et al., 1987; Cambronero et al., 1992; Harbuz et al., 1992). Glucocorticoids have potent immunosuppressive and anti-inflammatory effects (Barnes and Adcock, 1993). Most of these effects are explained by repression of the genes for cytokines. Dexamethasone induces the inhibition of interleukin-1 synthesis in the brain (Chai et al., 1996) and corticosteroids have been shown to inhibit central effects of interleukin-1 (Cambronero et al., 1989; Goujon et al., 1995; Chai et al., 1996). However, in contrast with the regulation of cytokine production are the effects of glucocorticoids on the expression of cytokine receptors. In fact the expression of many cytokine receptors is up-regulated by glucocorticoids (Wiegers and Reul, 1998).

The objective of the present study was to investigate whether i.c. inoculation of Theiler's virus to susceptible mice induces a regulation of hippocampal interleukin-1 receptors at different periods p.i. The effects of dexamethasone treatment on the regulation of interleukin-1 receptors in the hippocampus of mice following TMEV infection and the severity of induced encephalomyelitis symptomatology were assessed.

2. Materials and methods

2.1. Animals

Male and female SJL/J (strain susceptible to demyelination; 4- to 5-week old) from Jackson laboratories and male BALB/cByJ (strain resistant to demyelination) mice from our in-house colony (Cajal Institute, Madrid) were housed in groups of four to five and maintained on food and water ad libitum in a 12-h dark–light cycle (lights on from 7:15 AM to 7:15 PM).

2.2. Materials

[^{125}I]interleukin-1α (specific activity about 1400–1800 Ci/mmol) was purchased from DuPont–New England Nuclear (Boston, MA, USA). Unlabeled recombinant human interleukin-1α was a gift from Hoffmann–LaRoche (Nutley, NJ, USA). Dexamethasone was purchased from Fluka (Buchs, Switzerland).

2.3. Viruses and animal inoculation

The TMEV from a DA strain was a generous gift from Dr. Raymond Roos, Department of Neurology, University of Chicago Medical Centre. The viruses were derived from transfection of an infectious clone onto L929 cells (with plaque purification), receiving afterwards four passages in baby hamster kidney-21 cells. Infectious virus was titrated, aliquoted and stored at −80°C until use.

Mice were anesthesised with ethyl ether (Panreac, Barcelona, Spain) and inoculated in the right cerebral hemisphere on day 0 with 1×10^6 plaque formation units of TMEV in 30 μl of Dulbecco's modified Eagles medium containing glucose 4.5 g/l (Bio-Biowittaker, Verviers, Belgium) and supplemented with 200 nM glutamine (Flow, Nuclear Iberica, Madrid, Spain) and 10% fetal calf serum. Control SJL/J mice received the same volume of the vehicle. The injection site was a point two-thirds of the distance between the posterior edge of the right eye and right ear. Mice were examined once weekly for the first 3 weeks and three times weekly thereafter for development of neurological signs. The clinical score was graded as follows: 0, no gait abnormality; 1: waddling gait; 2: spastic hind limb paralysis; 3: paraplegia; 4: incontinence. These clinical scores have been shown to be indicative of demyelination (Lipton, 1975). Clinical data are expressed as the mean score of clinically affected animals at a particular time point.

2.4. Tissue preparation

The mice were decapitated, at different periods p.i. The brain was removed and the hippocampus quickly dissected on ice. Tissues were placed on ice-cold buffer and processed immediately. The buffer was RPMI 1640 (Flow, Irvine, Scotland, UK) supplemented with: 50 μg/ml gentamicin, 20 mM HEPES, 1 mg/ml sodium azide, 100 kallikrein inhibitor units/ml aprotinin, and 10^{-4} M bacitracin, pH 7.4. Tissues were homogenised with a motor-driven Teflon pestle (Heidolph, Germany) and glass-tube on ice, with 20 strokes at 600 rpm. The homogenate was centrifuged at $20\,000 \times g$ for 12 min at 4°C, and washed by resuspending the pellet in the same buffer and re-centrifuging with the same parameters. The tissue was then re-suspended in buffer to a final protein concentration of 100–133 μg/ml. The protein concentration of the membrane suspension was determined using a commercial kit (BCA Protein Assay Reagent, Pierce, Rockford, IL, USA), with bovine serum albumin (Sigma, St. Louis, MO, USA) as a standard. Binding of interleukin-1 receptors in the hippocampus was determined for each animal, except for saturation binding experiments, when pools of seven to eight hippocampi were used.

2.5. Interleukin-1 binding assay

The binding assay was performed according to the technique described elsewhere (Betancur et al., 1994). Briefly, incubation tubes contained 100 μl of [^{125}I]interleukin-1α (final concentration about 50 pM in individual point assays and approximately 1–200 pM in saturation studies), 100 μl of the incubation buffer (the same buffer used for tissue preparation supplemented with 0.15% bovine serum albumin), or an excess of unlabeled interleukin-1α (33 nM), to determine non-specific binding and 100 μl of membrane suspension (100–133 μg protein/tube). Binding equilibrium was reached after 3 h at room temperature. After incubation, unbound radioactivity was separated from the tissue by centrifugation at $12\,000 \times g$ for 5 min. The resulting pellet was then washed with 1 ml of ice-cold Dulbecco's phosphate-buffered saline without calcium chloride or magnesium chloride (Sigma), containing 0.01% Triton X-100 (Panreac), pH 7.2, and re-centrifuged at $12\,000 \times g$ for 5 min. The supernatant was aspirated and the remaining radioactivity was measured in a LKB gamma-counter (Wallak, Turku, Finland) at 75% efficiency.

2.6. Dexamethasone treatment

A set of experiments was designed to study the effect of dexamethasone treatment on interleukin-1 receptor binding modulation by TMEV inoculation. The animals were injected intraperitoneally with dexamethasone (500 μg/kg or 1 mg/kg) every 12 h from day 5 post-TMEV inoculation until day 10, where they were killed. Controls (TMEV–saline-treated mice) received the same volume of vehicle (saline with ethanol 2%).

2.7. Data analysis

The data are expressed as fentomoles of bound [^{125}I]interleukin-1α per mg of protein (mean ± S.E.M.). In saturation binding studies, maximum binding capacity (B_{max}) and equilibrium dissociation constant (K_d) were derived using the non-linear curve-fitting program Ligand© of Munson and Rodbard (1980). The significance of differences between means in two groups was calculated with Student's *t*-test. Analysis of variance (ANOVA) was used to assess the overall differences between three treatment groups, followed by Tukey's test for comparison between means. Comparisons of the percentage of mice exhibiting clinical disease between treatments were analysed by test, using Fisher's exact probability. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Interleukin-1 receptors in hippocampus of different strains of mice

Previous studies in our laboratory had established the optimum conditions to evaluate interleukin-1 binding in mouse hippocampus, using crude membrane homogenates

Table 1
[^{125}I]interleukin-1α (IL-1α) binding to hippocampus in different strains of mice

Strain	[^{125}I]IL-1α bound (fmol/mg protein)
BALB/c	1.041 ± 0.05
Swiss	1.160 ± 0.14
C3H/He	0.942 ± 0.24
SJL/J	1.330 ± 0.08
C57/BL6	1.102 ± 0.10

Values are means ± S.E.M. from four to five experiments.

(Betancur et al., 1994). Human [^{125}I]interleukin-1α showed similar values for specific binding in different mouse strains. Interleukin-1 binding sites were very similar in the hippocampus of Swiss and C57/BL6 mice (Table 1). BALB/cByJ male and female mice had similar values for specific binding in hippocampal membrane preparations (males: 0.86 ± 0.06 fmol/mg protein; females: 1.17 ± 0.16 fmol/mg protein). However, SJL/J males had a significantly higher [^{125}I]interleukin-1α binding to hippocampal membranes than SJL/J females (males: 1.45 ± 0.11 fmol/mg protein; females: 0.83 ± 0.07 fmol/mg protein). We used male SJL/J and BALB/cByJ mice for subsequent experiments.

3.2. Time course effects of TMEV infection on hippocampal interleukin-1 receptors

In the next group of experiments we assessed interleukin-1α binding to hippocampus of TMEV infected mice. Fig. 1 shows that infection with TMEV resulted in differences in interleukin-1α binding at 4, 10, 14, and 30 days post-inoculation. A significant decrease in interleukin-1 binding was observed at 10 days (controls: 0.648 ± 0.062 fmol/mg protein; TMEV: 0.280 ± 0.037 fmol/mg protein; $P < 0.001$) and 14 days (controls: 0.453 ± 0.049 fmol/mg protein; TMEV: 0.264 ± 0.028 fmol/mg protein; $P < 0.01$) post-inoculation. There were no differences in specific binding values between infected and control animals 4 and 30 days p.i. Similarly, no differences in interleukin-1 binding between TMEV and sham-infected mice were detected 40 and 100 days p.i. (data not shown).

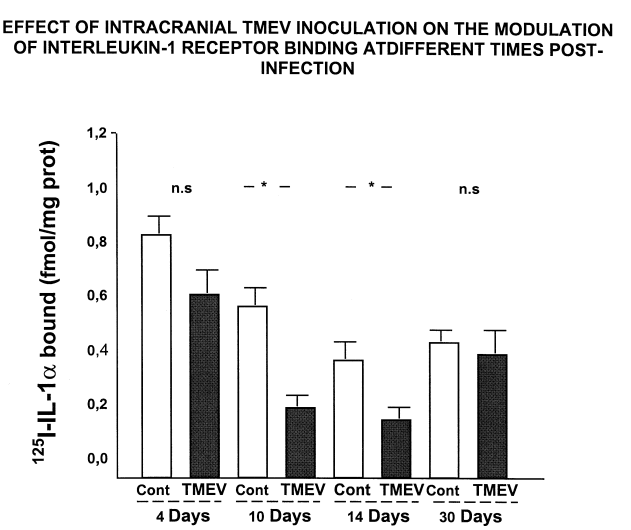

Fig. 1. Time course effects of TMEV infection on [^{125}I] recombinant human interleukin-1 binding in mouse hippocampus. Mice were injected intracranially either with TMEV or vehicle, and killed at different time intervals p.i.: 4 days (control: $n = 10$; TMEV $n = 10$), 10 days (control: $n = 10$; TMEV: $n = 10$), 14 days (control: $n = 7$; TMEV: $n = 7$), 30 days (control: $n = 8$; TMEV: $n = 8$). Interleukin-1 receptors were assayed by incubating hippocampal membranes from individual animals with [^{125}I]interleukin-1α (50 pM). Statistical analysis revealed a significant difference for control mice vs. TMEV-infected mice at 10 ($P < 0.001$) and 14 days ($P < 0.01$) p.i.

3.3. Effect of TMEV infection on hippocampal interleukin-1 receptors of susceptible and resistant mouse strains

We then tried to find if changes in interleukin-1 receptor binding were unspecific phenomena or were related to the susceptibility of the animals to develop the disease. Fig. 2 shows the results of interleukin-1 binding in strains of mice sensitive and resistant to TMEV encephalomyelitis 10 days p.i. BALB/cByJ mice had not changes in the values of interleukin-1α binding after TMEV injection, contrary to what was shown for SJL/J mice. The difference between both groups was highly significant ($P < 0.001$).

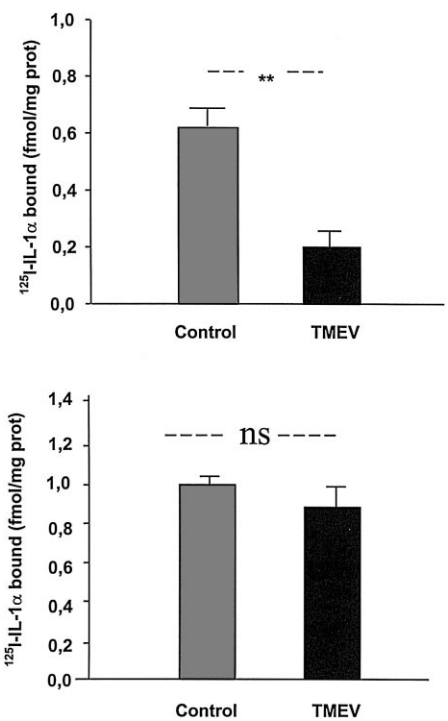

Fig. 2. Comparison of effects of intracranial TMEV inoculation on specific [^{125}I] recombinant human interleukin-1α binding in hippocampus in susceptible (SJL/J) and resistant (BALB/c) mouse strains. Values are the means ± S.E.M. for ten to six animals per group. ***$P < 0.001$ TMEV vs. control mice (susceptible mice).

Interleukin-1α saturation binding experiments were performed with TMEV-infected and control animals, to determine whether the TMEV-induced decreases we observed 10 and 14 days p.i. were due to alterations in density (B_{max}) and/or affinity (K_d) of the interleukin-1 receptors in the hippocampus. A representative Scatchard plot of [^{125}I]interleukin-1α binding in infected and control animals is shown in Fig. 3. The saturation data revealed that the decrease in interleukin-1 binding was due to partly a decrease in the number of interleukin-1 receptors but, mainly, to complete loss of the affinity of the interleukin-1 receptors for its ligand (controls: $B_{max} = 2.18$ fmol/mg protein and $K_d = 10.59$ pM; TMEV infected: $B_{max} = 0.84$ fmol/mg protein and $K_d = 1.32$ pM).

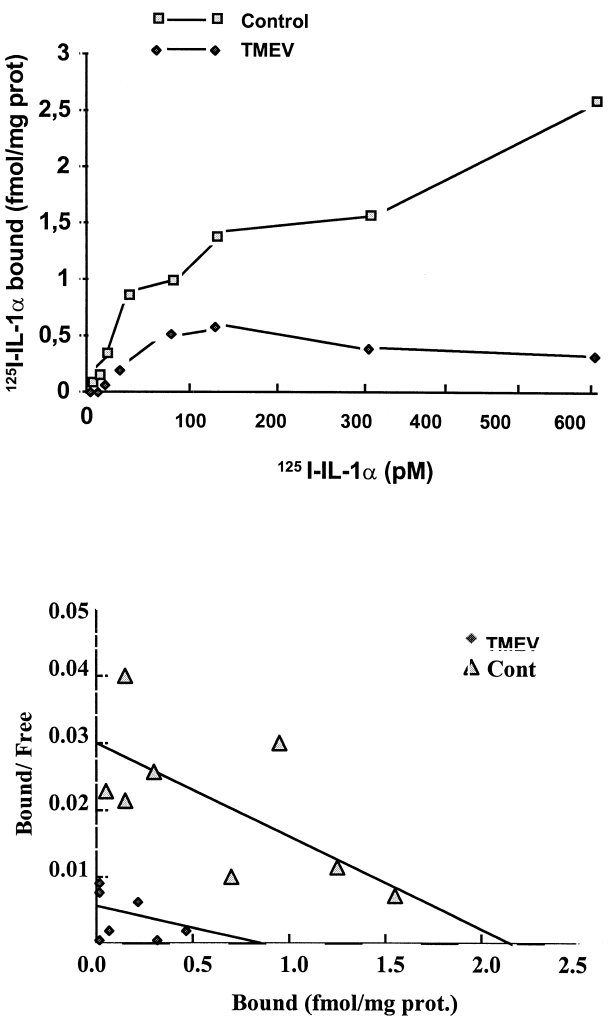

Fig. 3. Binding of [^{125}I]interleukin-1α in the hippocampus of control or TMEV infected SJL/J mice as a function of increasing ligand concentration (2–600 pM). Scatchard plot of [^{125}I]interleukin-1α specific binding to hippocampal membranes from TMEV or vehicle-treated mice. The data shown are from a representative experiment. Control mice: B_{max} = 2.18 fmol/mg protein; $K_d = 10.59$ pM. TMEV-infected mice: B_{max} = 0.84 fmol/mg protein; $K_d = 1.32$ pM.

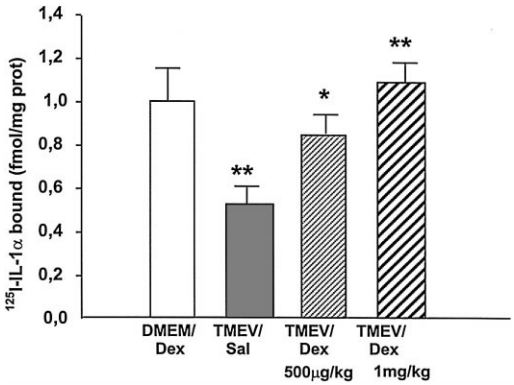

Fig. 4. Effect of dexamethasone treatment on specific [^{125}I]interleukin-1α binding in the hippocampus of TMEV-infected mice at 10 days p.i. Mice were injected intraperitoneally every 12 h for days 5 to 10 p.i. with dexamethasone (0.5 or 1 mg/kg) or vehicle. Data represent means ± S.E.M. for six to eight animals per group. $^{*}P < 0.05$; $^{**}P < 0.01$

3.4. Effect of dexamethasone treatment on TMEV-induced modulation of interleukin-1 receptors

In the last group of experiments we studied the effects of dexamethasone administration on TMEV-induced modulation of interleukin-1 receptors at 10 days p.i. As shown in Fig. 4, interleukin-1 binding was significantly decreased in TMEV-treated mice, but this down-regulation was significantly reversed by the administration of dexamethasone 500 μg/kg or 1 mg/kg from days 5 to 10 p.i. ($F_{3,28} = 12.45.6$, $P < 0.01$; Tukey *B*-test: TMEV/saline < TMEV/dexamethasone (500 μg/kg) $P \leq 0.05$; TMEV/saline < TMEV/dexamethasone (1 mg/kg) $P \leq 0.01$).

3.5. Effect of dexamethasone treatment on clinical signs of TMEV-induced demyelinating disease

To determine the effects of dexamethasone treatment on the severity of the demyelinating disease, TMEV-infected mice were treated with the glucocorticoid according to the same schedule as above and observed for clinical symptoms. Fig. 5 illustrates experiments, with eight animals per group, representative of the clinical course of TMEV encephalomyelitis after dexamethasone treatment. Dexamethasone reduced the incidence (shown in parentheses) and clinical severity, as well as delayed the onset of disease compared with results for TMEV/saline-mice. On day 40 p.i., disease incidence was six out of eight for the TMEV/saline group and zero out of eight for the TMEV/dexamethasone (500 μg/kg) and TMEV/dexamethasone (1 mg/kg) ($P < 0.01$, by χ^2 analysis). On day 60 p.i., disease incidence was eight out of eight for the TMEV/saline group, three out of eight for the TMEV/dexamethasone (500 μg/kg) and zero out of eight for the TMEV/dexamethasone (1 mg/kg) group. These results suggest strongly that dexamethasone treatment in

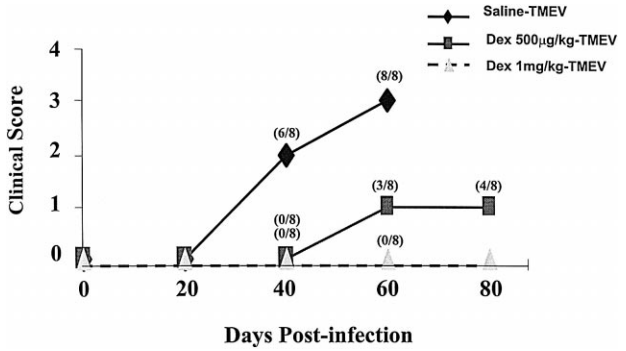

Fig. 5. Effect of dexamethasone treatment on the course of the disease. Groups of eight mice were given intracranially 1×10^6 PFU of TMEV at day 0. Two groups of mice were given dexamethasone (500 μg/kg or 1 mg/kg, twice/day i.p.) and the other group received vehicle from day 5 to day 10 p.i. Disease severity was assessed at different time intervals p.i., by grading neurological signs described in Section 2. The data represent the mean clinical score of the group over time following TMEV infection.

the early stages of TMEV infection is able to prevent clinical symptomatology.

4. Discussion

We now showed that interleukin-1 receptors in the mouse hippocampus are modulated during TMEV infection. The regulation of interleukin-1 binding is time-dependent on the viral p.i. period. There is a decrease in interleukin-1α binding in the murine hippocampus at 10 and 14 days p.i., which correlates with the accumulation of T-cell and macrophage infiltrates at this time (Lindsley and Rodriguez, 1989). Two explanations could account for decreased interleukin-1 binding: the first is that, as reported by Wada and Fujinami (1993), TMEV-infected cells in the hippocampus elicit an influx of inflammatory cells (monocytes/macrophages), but also of astrocytes and microglia, which could produce substantial amounts of interleukin-1 during this acute phase. The presence of interleukin-1 could modulate the expression of the receptor. Second, it has been reported (Tsunoda et al., 1997) that TMEV can induce apoptosis of hippocampal neurones. Thus, after infection, fewer neurones in the hippocampus would bind less labeled interleukin-1. However, this second possibility seems to be less probable, since the treatment with dexamethasone reversed the decrease in interleukin-1 binding. It is important to note that the reduction in interleukin-1α binding in the murine hippocampus 10 days after TMEV inoculation was observed only in susceptible SJL/J mice but not in resistant BALB/c. This could suggest that, in susceptible mice, the possible endogenous interleukin-1 production by TMEV infection may act within the brain to down-regulate its own receptor. Similar down-regulation of interleukin-1 receptor has been shown in monocytes and fibroblasts and on a glioblastoma cell line (Mizel et al., 1987; Gottschall et al., 1991). The early appearance of cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor-α has been documented in TMEV infection (Rubio and Torres, 1991; Sato et al., 1997) as well as in other CNS viral infections (Wesselingh and Griffin, 1994), and may be related, as mentioned before, to their secretion by a variety of cells of both immune and CNS origin (Benveniste, 1992). For example, rabies virus infection has been associated with down-regulation of brain interleukin-1 receptors (Marquette et al., 1996a), due to increased interleukin-1 levels in the mouse brain by activation of microglial cells and macrophages (Marquette et al., 1996b).

Our saturation experiments revealed that the TMEV-induced down-regulation of interleukin-1 receptors could be accounted for by a decrease in receptor density, but mainly by loss of receptor affinity. However, the reduction in interleukin-1 binding by lipopolysaccharide treatment has also been attributed to changes in receptor density (Ban et al., 1991; Takao et al., 1993). Therefore, based on the present results we cannot discard the possibility that the virus infection may modify the functional mechanisms of interleukin-1 receptor (pre-coupling of interleukin-1 receptors to second messengers system, protein phosphorylation, etc.) changing the affinity of interleukin-1 receptor for its endogenous ligand.

Several of the immune factors of resistance (Monteney et al., 1997) are controlled by the cytokine response to infection, and differences in cytokine profiles may be particularly relevant to susceptibility/resistance to the persistent infection. Thus the present report, provides evidence that, early after TMEV infection, only a susceptible mouse strain showed a reduction in interleukin-1α binding to hippocampal interleukin-1 receptor. These results are consistent with other data showing that astrocytes from TMEV-susceptible mice (SJL/J) are able to produce interleukin-1α but astrocytes from resistant Balb/c mice are not (Rubio and Capa, 1993). It has been reported that lipopolysaccharide treatment of TMEV-infected mice results in demyelinating disease in the normally resistant C57BL/6 mice (Pullen et al., 1995). Interestingly, in the above study (Pullen et al., 1995), the intraperitoneal administration of interleukin-1 was able to mimic the LPS effect. Therefore, production of interleukin-1 may be critical for an increased pathogenesis in SJL/J mice when compared with resistant Balb/c mice. However, the expression of interleukin-1 mRNA by RT–PCR in the brain of different strains of mice appears to be similar at early times p.i. with TMEV (Sato et al., 1997).

Reciprocal interactions between glucocorticoids and interleukin-1 receptors have been described in several studies (Wiegers and Reul, 1998). As an example, interleukin-1 down-regulates glucocorticoid receptors in rat hippocampus (Weidenfeld et al., 1989), but previous work in our laboratory using specific ligands of corticosteroid receptors, showed a lack of effect of central administration of

interleukin-1 on type I and type II corticosteroid receptors in the rat hippocampus (Betancur et al., 1995). However, glucocorticoids have been shown to regulate the expression of interleukin-1 receptors. Incubation with dexamethasone decreases interleukin-1 binding in AT-20 pituitary tumor cells (Kobayashi et al., 1992). Conversely, glucocorticoids enhance the expression of interleukin-1 receptors on several cell types (Akahoshi et al., 1988; Gottschall et al., 1991). In the present study, dexamethasone treatment in the early phase of TMEV infection (days 5 to 10 p.i.) reversed the TMEV-induced down-regulation of hippocampal interleukin-1 receptors. This may be interpreted as a decrease in the endogenous production of interleukin-1 following TMEV infection, due to the glucocorticoid treatment. As mentioned earlier, corticosteroids inhibit the transcriptional and post-transcriptional expression of the interleukin-1 gene and decrease the stability of interleukin-1 mRNA (Knudsen et al., 1987; Lee et al., 1988), and adrenalectomy enhances interleukin-1 gene expression in the brain of mice given lipopolysaccharide (Goujon et al., 1996). Another possible explanation is that dexamethasone up-regulates the expression of interleukin-1 receptors in the hippocampus, as observed with other cell types. Nevertheless, we have shown that hippocampal interleukin-1 receptors are relatively resistant to modulation by varying levels of circulating corticosteroids (Betancur et al., 1994). It should be noted that the administration of dexamethasone (days 5–10 p.i.), not only prevented the decreased interleukin-1 binding but also suppressed neurological manifestations of TMEV-demyelination. These observations suggest that CNS endogenous production of interleukin-1 in the acute phase of the disease seems to be critical for later chronic demyelination following TMEV infection. It would also be interesting to evaluate whether the sensitivity of SLJ/J mice to Theiler virus-induced demyelination could be associated with an impaired reactivity of the hypothalamic–pituitary–adrenocortical axis in this mouse strain to viral infection, as many studies have shown that alterations in hypothalamic–pituitary–adrenal responses play an important role in the susceptibility to inflammatory/autoimmune disease (Wick et al., 1993).

The putative mechanisms through which interleukin-1 could influence TMEV-induced demyelination disease remain to be elucidated. However, it is important to note that interleukin-1 activates $CD4^+$ cells to produce interleukin-2 and enhances the expression of interleukin-2 receptors in T-cells. Interleukin-1 activates B-cells, but also glial and endothelial cells (Benveniste, 1992). Indeed, interleukin-1 may lead to an increase in the expression of adhesion molecules (Fabry et al., 1992) which, in turn, favours the recruitment of T-cells and macrophages. Interestingly, TMEV-infected cerebrovascular endothelial cells have a four- to five-fold increase of interleukin-1β mRNA levels over non-infected cells (Sapatino et al., 1995).

Based on our data, we suggest that the suppression of the TMEV-induced reduction in interleukin-1α binding in the early phases of infection may lead to prevention of the demyelinating disease. The protective effect of corticosteroids has been demonstrated in a number of studies (MacPhee et al., 1989; Whitaker et al., 1993). The present results support this suggestion, and emphasise the importance of the pro-inflammatory cytokine interleukin-1 in the early pathogenic events in TMEV-induced demyelination.

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