The efficacy of Cyclosporin A, FK-506 and Prednisolone to modify the adoptive transfer of Experimental Allergic Encephalomyelitis (EAE)

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Abstract
The in vitro potency of the immunosuppressants Cyclosporin A (CsA), FK-506 and Prednisolone was assessed using the adoptive transfer model of EAE in the Lewis rat. Co-culture of encephalitogen-sensitised splenic leukocytes with Prednisolone did not inhibit the transfer of disease to naive histocompatible recipients despite significant suppression of neuroantigen-stimulated leukocyte proliferation by the drug. The addition of CsA (100 nM) to cultures inhibited the induction of adoptive EAE but a lower dose of the agent (10 nM) did not prevent the development of clinico-histopathological signs of disease. FK-506 (1 nM) was 100 times more effective than CsA at suppressing adoptive EAE thus emphasising the usefulness of the model in determining the relative efficacy of compounds to modify cell-dependent autoimmune disease.

Introduction
The initial observation by Paterson [1] that the autoimmune disease Experimental Allergic Encephalomyelitis (EAE) could be induced by the transfer of lymphocytes from actively-sensitised rats to naive histocompatible recipients confirmed the condition to be, principally, an immune cell-mediated phenomenon. Subsequent studies revealed the specific requirement of antigen-presenting macrophages and T-cell subsets for the successful mediation of EAE [2, 3, 4]. Furthermore, work by Panitch and McFarlin [5] and Richert et al. [6] demonstrated that adoptively-transferred lymphocytes, when pre-incubated with either the polyclonal mitogen Concanavalin A or the neuroantigen myelin basic protein (MBP), had an improved capacity to generate disease. EAE has become well established as an animal counterpart for the human central nervous system (CNS) disease Multiple Sclerosis (MS) [7] and has contributed to a clearer understanding of the mechanisms which may underlie the etiology of demyelination [8]. In addition, pharmacological studies utilising both the active and adoptive models of EAE have provided useful information on the mechanisms by which steroidal and non-steroidal immunomodulatory drugs may act and be of potential value in the treatment of MS [9–11]. Indeed, the rationale for instigating clinical trials with the immunosuppressive compound Cyclosporin A (CsA) originated from extensive work investigating the efficacy of the drug in various models of EAE [12, 13]. Recently the novel macrolide antibiotic FK-506 was shown to prevent or significantly modify the course of actively-induced EAE in the Lewis rat [14] and additional studies demonstrated the drug to be effective in other experimental models of autoimmunity [15–18]. The present study exam-
ines the effects of FK-506 on the adoptive transfer of EAE and determines the potency of the drug relative to CsA and the steroidal compound Prednisolone.

**Materials and methods**

**Animals**

Male Lewis rats, weighing 200–225 g, were obtained from Bantin and Kingman Ltd, Hull, UK, and maintained on Labsure CRM rat diet and water ad libitum.

**Induction of active EAE**

Animals received, in each hind footpad, 0.2 ml of a water-in-oil emulsion containing 50 μg of solubilised MBP, prepared by the method of Dunkley and Carnegie [19] and an equal volume of Freund’s complete adjuvant containing 5 mg/ml Mycobacterium tuberculosis H₃₇Ra (Difco, Surrey, UK).

**Induction of adoptive EAE**

The adoptive transfer of disease was performed as previously described by Bolton et al. [9]. Briefly, spleens were aseptically removed from rats showing paralytic signs of EAE, 13 days post-inoculation, and pooled single cell suspensions were prepared in RPMI 1640 medium containing 5% heat-inactivated, mycoplasma-screened foetal calf serum, 2 mM glutamine, 2 × 10⁻⁵ M 2-mercaptoethanol, 100 units penicillin and 100 μg streptomycin/ml⁻¹ (Gibco Ltd, Uxbridge, UK). Viable leukocytes, as assessed by trypan blue exclusion, at a concentration of 2 × 10⁶ cells/ml⁻¹ were cultured in the presence of 1 μg/ml⁻¹ MBP for 72 hrs at 37°C in a humidified atmosphere of 5% CO₂. Harvested cells were washed thoroughly in Hank’s balanced salt solution and 2–4 × 10⁷ viable leukocytes were injected, via the tail vein, into each histocompatible recipient.

**In vitro determination of MBP-induced proliferation**

At the initiation of culture 200 μl aliquots were removed from cell preparations, dispensed in groups of five in round-bottom microculture plates and incubated as above. Cells were pulsed with 1 μCi ³H-thymidine per well (specific activity 5.0 Ci/mmol) 24 hr prior to harvesting and radionucleotide uptake was quantitated using an LKB-Wallace Liquid Scintillation Counter.

**Assessment of neurological EAE**

The body weight and neurological status of each animal was recorded daily. The day on which clinical signs of EAE developed in each rat was noted and symptoms were graded as follows: 1. flaccid tail; 2. hind limb hypotonia; 3. partial hind limb paralysis; 4. complete hind limb paralysis.

**Assessment of histological EAE**

Cervical spinal cords were removed from animals, following complete loss of symptoms, and fixed in 10% formal saline. Sections, 10 μ thick, were cut, stained in haematoxylin-eosin, coded to prevent identification and the presence or absence of perivascular lesions was determined by light microscopy.

**Drugs**

Prednisolone (Sigma, UK), CsA (Sandoz Ltd, Switzerland) and FK-506 (Fujisawa Pharmaceutical Co. Ltd., Japan) were solubilised in absolute alcohol, diluted to the required concentrations in complete medium and added, simultaneously with MBP, to preparations at the initiation of culture.

**Statistical analysis**

Students t-test was used to assess differences between control and drug treatments.

**Results**

**Drug effects on the adoptive transfer of EAE to naïve recipients**

Splenic leukocytes cultured in the presence of MBP and injected into recipient rats induced neurological and histological signs of EAE whereas cells incubated without the encephalitogen did not transfer disease (Table 1). The addition of 1 μM Prednisolone to MBP-stimulated cultures proved to be cytotoxic as assessed by the trypan blue exclusion test (data not shown). A lower concentra-