122P    EFFECT OF AN ALPHA4 INTEGRIN BLOCKING ANTIBODY ON NEUROINFLAMMATION AND ACUTE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE LEWIS RAT

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The invasion of leukocytes into the CNS is a key feature in the pathogenesis of multiple sclerosis (MS) and the animal model, experimental allergic encephalomyelitis (EAE). The cell adhesion molecules alpha4beta1 (α4β1, VLA-4) and alpha4beta7 (α4β7, LPAM-1) have been shown to play a pivotal role in this process (Leone et al., 2002; Kanwar et al., 2000). In this study we have used a blocking antibody to examine the role of alpha4 integrins in two models of CNS inflammation; intracerebral TNF injection and actively induced EAE.

Female Lewis rats (175 – 225 g) were pre-treated (-24 hr) with either MAX68P (mouse anti-human α4 antibody; IgG1) or 101.4 (isotype matched negative control antibody, 10 mg kg⁻¹; s.c). Rat recombinant TNFα (1 µg) was injected into the left striatum of rats anaesthetised using isofluorane / N₂O, according to the method of Schnell et al. (1999). Twenty-four hours later animals were sacrificed and transcardially perfused with saline, the brains were embedded in cryoprotectant medium & frozen in isopentane. Infiltrating cells were identified immunohistochemically using the rat macrophage ED1 marker and the number of positive cells per mm² determined by microscopy and image analysis (KS300, Image Associates).

Female Lewis rats (175 – 225 g) were immunised, at the base of the tail, with an intradermal injection of complete Freund’s adjuvant (100 µl) containing 2 mg ml⁻¹ Mycobacterium & 150 µg ml⁻¹ of guinea pig myelin basic protein. Rats were treated prophylactically (from day -1) with MAX68P or 101.4 (10 mg kg⁻¹; s.c; twice weekly). Disease severity was assessed daily by a modification of the standard method (Leone et al., 2002).

MAX68P caused a significant reduction in the number of TNFα-dependent, striatal ED1 positive cells per mm² (372.6 ± 42.7 & 84.7 ± 35.6 for 101.4 & MAX68P respectively; mean ± SD, n=6, P<0.0001, students t test). In Lewis rat EAE, MAX68P caused a significant delay in onset of disease compared with isotype treated controls (101.4: day 10 ± 0.5, MAX68P: day 12.6 ± 1.1, mean ± SD, n=15, P<0.0001, Mann Whitney U test). No significant differences were observed in disease severity or incidence.

These results indicate that in the rat, leukocyte infiltration in response to an intrastrial injection of TNFα is dependent on alpha4 integrins. In EAE, blocking alpha4 integrins delayed the onset of disease without altering disease severity. This suggests that in this model of MS alpha4 integrins play a significant early role, whilst other mechanisms may mediate leukocyte trafficking later in the disease course.