

Effect of CC chemokine receptor 4 antagonism on the evolution of experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS) wherein T helper (Th) 17 cells are the major pathogenic players orchestrating demyelination of axons. Recently in PNAS, Poppensieker et al. (1) documented a key role on the requirement of CC chemokine receptor 4 (CCR4) in EAE. The authors reported that CCR4 is required for GM-CSF-dependent IL-23 secretion by dendritic cells (DCs) in the CNS and for the maintenance of Th17 cells and the development of EAE. The results thus indicate that CCR4 is a potential therapeutic target in EAE, and eventually MS.

Therapeutic strategies to target DC-specific CCR4 are not yet available. However, several functionally potent small molecule antagonists to CCR4 in general, irrespective of its expression on cell types, have been identified (2, 3). Therefore, we explored the therapeutic utility of antagonizing CCR4 in EAE.

We induced EAE in C57BL/6 mice in three different groups by injecting myelin oligodendrocyte glycoprotein (MOG_{35–55}) in complete Freund's adjuvant. On the day of onset of clinical signs [day (d) 8], a group of mice were injected daily with 1.5 μg of a CCR4 antagonist, AF399/420/18025 (4-(1-benzofuran-2-ylcarbonyl)-1-{5-[4-chlorobenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}-3-hydroxy-5-(2-thienyl)-1,5-dihydro-2H-pyrrol-2-one; Formula-C₂₆H₁₆ClN₃O₄S₃; molecular weight 565.93), for 5 d by intraperitoneal route. The dose of CCR4 antagonist was chosen based on our previous studies (2, 4). Mice in the control group developed clinical signs from d8 onwards, reaching a mean maximal score of 2.5 on d20 (Fig. 1A). However, the group of mice with EAE that were treated with CCR4 antagonists did not show any significant reduction in the clinical score, and the disease pattern remained the same as that of controls.

To explore whether prophylactic use of CCR4 antagonists would ameliorate clinical signs of EAE, a group of mice were injected with CCR4 antagonists from d0 to d4. In contrast to therapeutic use of CCR4 antagonists, mice that received prophylactic injections of CCR4 antagonists showed an exaggerated disease pattern with the clinical scores close to statistical significance compared with control mice ($P = 0.07$) (Fig. 1A). Further, we did not observe any significant differences in the mean maximal score of EAE in controls and the two treated groups (Fig. 1B). Also, the incidence of EAE was the same with or without CCR4 antagonism (Fig. 1C).

These results suggest that antagonizing CCR4 in general without cell specificity has no beneficial effect in EAE. The

possible reason could be that CCR4 is also expressed by CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs). In mice, 15–20% of bona fide Tregs express CCR4 (4). Tregs are implicated in the recovery from EAE and in the suppression of encephalitogenic T cells and innate cells, such as DCs and macrophages. The increased severity of clinical signs in the prophylactic group might be attributable to inhibition of Treg migration toward DCs in the draining lymph nodes, leading to enhanced activation of naive T cells and their differentiation into Th17 cells (2, 4). However, in contrast to mouse Tregs, 75–90% of human Tregs express CCR4 (5); hence, broad CCR4 antagonism in humans might further aggravate the disease and pathogenesis. Although we do not challenge the concept of DC-specific CCR4 antagonism, our data suggest that results from the genetic deletion experiments in mice must be cautiously interpreted for translation into therapeutic setup.

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Conflict of interest statement: J.B. holds a patent on the use of a CCR4 antagonist as an adjuvant, specifically in an immunogenic composition comprising an antigen that elicits an immune response against a pathogen or tumour (WO2009150433, European and US patent).

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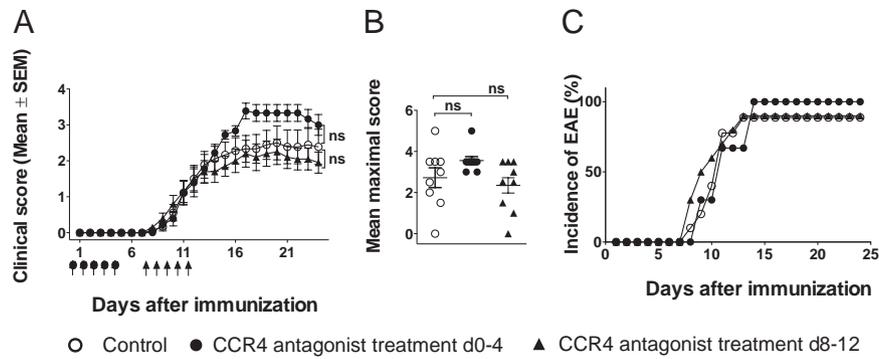


Fig. 1. Antagonizing CCR4 does not affect the course and severity of EAE. Ten-week-old female C57BL/6J mice (Janvier Laboratories) were immunized with 200 μ g of myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅; MEVGWYRSPFSRVVHLYRNGK) peptide in complete Freund's adjuvant containing 500 μ g of nonviable *Mycobacterium tuberculosis* (H37RA). Three hundred nanograms of pertussis toxin (List Biologic Laboratories) in PBS was injected i.v. on the day of immunization and 48 h later. All animal studies were performed according to the guidelines of the Charles Darwin Ethical Committee for Animal Experimentation (Université Pierre et Marie Curie) at the pathogen-free animal facility of the Centre de Recherche des Cordeliers. The CCR4 antagonist AF399/420/18025 was dissolved in DMSO and the final solution for injection was prepared in 10% DMSO to maintain the solubility of the antagonist. Mice in the CCR4 antagonist treatment group received 1.5 μ g of antagonist in 100 μ L volume injected i.p. daily either from d0 to d4 (prophylaxis regimen, ●) or after onset of clinical signs according to the following scoring pattern: 0, no signs; 1, tail paresis; 2, hind limb paresis; 3, hind limb paralysis; 4, tetraplegia; and 5, moribund. Error bars represent SEM. (A) Daily mean clinical scores of each group (9–10 mice per group; $P > 0.05$, two-way ANOVA with Bonferroni post t test). ns, nonsignificant. (B) Mean maximal score of each group during the entire period of study ($P > 0.05$, Mann–Whitney U test). (C) Percentage incidence of EAE in each group plotted against time.