



CCR9 inhibition does not interfere with the development of immune tolerance to oral antigens

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ABSTRACT

Recent literature indicates that mice deficient in the chemokine receptor CCR9 (CCR9^{-/-} mice) are unable to generate oral tolerance. The present report describes how such inability can be overcome by increasing the dose of oral antigen. Pharmacological inhibition of CCR9 did not affect the generation of oral tolerance, regardless of antigen dose. These results highlight the inadequacy of genetic deletion of CCR9 when predicting the effects of pharmacological CCR9 inhibition on intestinal biology.

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1. Introduction

CCR9 is expressed on leukocytes, including T cells [1], B cells [1] and dendritic cells (DC) [2], and plays a central role in their migration to the intestines and thymus. CCL25, the only known ligand for CCR9, is expressed by intestinal and thymic epithelial cells [3]. Indeed, interference with the CCR9/CCL25 axis results in decreased antigen-specific T cell recruitment to the intestine [4]. CCR9 expression has also been reported on immature plasmacytoid dendritic cells (pDC), which play a role in the generation of immune tolerance [5]. Cassani et al. [6] reported that CCR9^{-/-} mice had a diminished capacity to generate immune tolerance to oral antigens. Results from the recent PROTECT-1 clinical trial in Crohn's disease showed that the CCR9 antagonist CCX282-B (vercirnon) was well tolerated and efficacious [7]. Previously, treatment with the CCR9 antagonist CCX282-B was shown to be therapeutic in the TNF-ΔARE mouse model of ileitis [8]; however, CCR9^{-/-} TNF-ΔARE mice displayed a different phenotype than those treated with compound [9,10]. Because of these known differences between genetic deletion and pharmacological inhibition, the ability of a CCR9 antagonist to modulate immune tolerance to oral antigen was tested.

2. Methods and materials

All animal experiments were approved by the ChemoCentryx Institutional Animal Care and Use Committee. Adoptive transfer and oral tolerance experiments are outlined in Figs. 1A and 2A, respectively. CCR9^{-/-} mice were obtained, under license, from Dr. Paul Love at the National Institute for Health. CCX507 was supplied by the Medicinal Chemistry Department at ChemoCentryx (Mountain View, CA). The effectiveness of CCX507 to block in vivo migration of antigen specific T cells to the small intestine was determined using an adoptive transfer model based on the methods outlined by Wurbel et al. [4]. T cells were isolated from CD45.2+ OT-1 mice and adoptively transferred into CD45.1+ mice. 24 h post transfer, mice were dosed with CCX507 or vehicle, and then orally challenged 1 h later with ovalbumin (OVA, Sigma, St. Louis, MI) alone or in combination with cholera toxin (Ctx; EMD Millipore, Billerica, MA). The small intestine was harvested 4 days later and the intraepithelial lymphocytes (IEL) isolated. IELs were stained with propidium iodide, CD45.2, CD45.1 and CD8 (BD Biosciences, San Jose, CA). The frequency of CD8+ CD45.2+ PI⁻ cells was determined in each IEL sample.

Oral tolerance was induced by orally dosing C57BL/6 mice with either 2.5 mg/day or 25 mg/day of OVA in PBS for 5 consecutive days. Mice orally dosed with PBS only were used as controls. All mice were immunized with 200 μg OVA/complete Freund's adjuvant (CFA, Chondrex, Redmond, WA) emulsion (250 μg OVA in saline plus 100 μl CFA) on day 7. On day 14, mice were challenged intradermally with OVA (500 ng) in the right ear and with saline in

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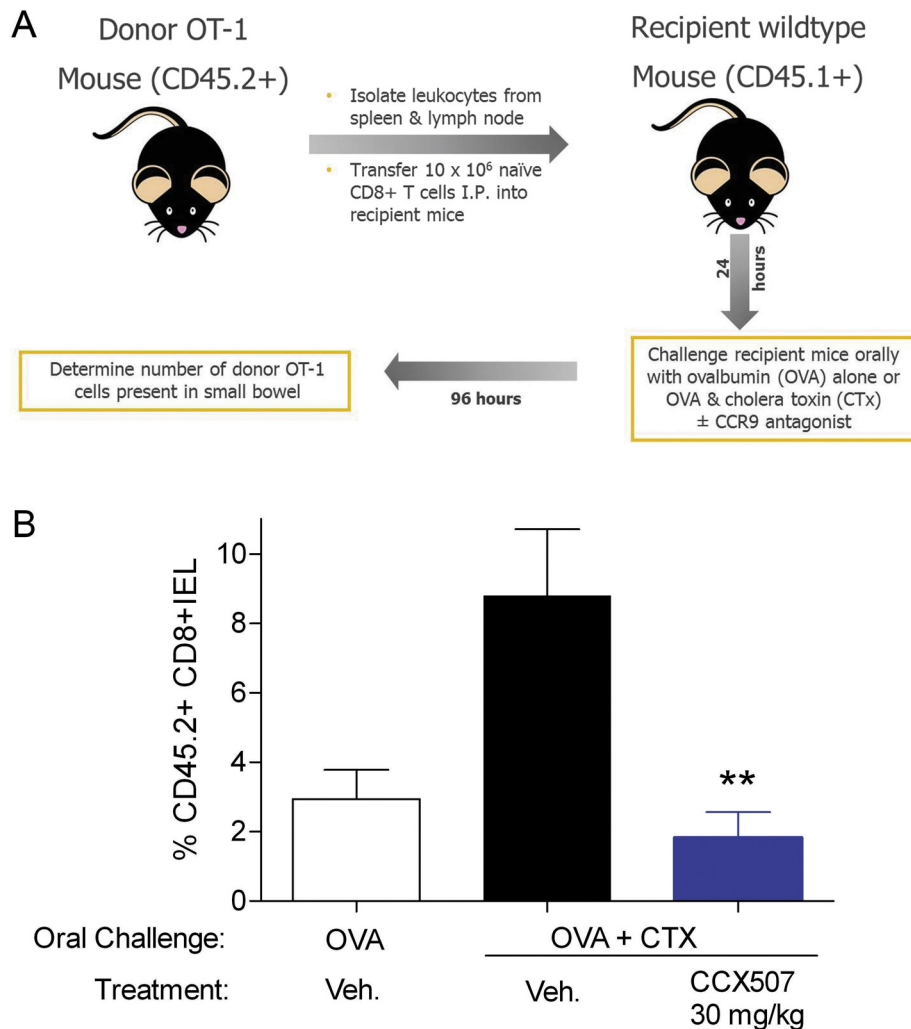


Fig. 1. Pharmacological blockade of CCR9 results in a significant reduction in T cell recruitment to the intestine. (A) Protocol for adoptive transfer model. (B) Adoptive transfer of CD45.2+ OT-1 T cells and subsequent challenge of recipient mice with OVA plus CTx results in a significant increase in CD45.2+ cell frequency within the small intestine (black bar). Antagonism of CCR9 with CCX507 (30 mg/kg; blue bar) significantly reduces accumulation of CD45.2+ cells to levels equal to animals challenged with OVA only (open bar). $n = 5-10$ animals per group, representative data shown from 2 separate experiments. ** $p < 0.01$ Mann Whitney test vs. appropriate control.

the left ear (Fig. 1A). Specific delayed type hypersensitivity (DTH) was calculated as the difference in thickness between the right and left ears 24 h post challenge. Although the earlier report [6] also utilized a DTH reaction as a measure of immune tolerance, the anatomical location used, the footpad, is different from that used in these studies. CCX507 (30 or 300 mg/kg) or vehicle was given prior to the first oral OVA exposure and dosed daily throughout the oral OVA challenges.

3. Results and discussion

The ability of the CCR9 antagonist CCX507 to block antigen-specific in vivo T cell migration was confirmed using an adoptive transfer model (Fig. 1A) [4]. Recipient mice challenged with OVA:CTx exhibited a significant increase in CD8+ CD45.2+ OT-1 cell frequency in the small intestine relative to mice challenged with OVA alone (Fig. 1B, $p = 0.01$). CCX507 (30 mg/kg) significantly reduced the frequency of CD45.2+ IEL in mice challenged with OVA:CTx (Fig. 1B, $p = 0.002$).

In studies to determine the effect of CCR9 inhibition on the generation of immune tolerance to oral antigens, C57BL/6 mice that received either 2.5 mg/day or 25 mg/day of oral OVA developed the expected systemic tolerance, as indicated by a significant decrease in antigen-specific ear swelling upon re-challenge (Fig. 2B and C;

$p < 0.01$ for both OVA groups vs. saline control). Consistent with the earlier report [6], CCR9^{-/-} mice receiving 2.5 mg/day oral OVA did not develop systemic tolerance to OVA, as evidenced by the ear swelling response upon re-challenge (Fig. 2B; no significant difference relative to control). However, challenge of CCR9^{-/-} mice with 25 mg/day oral OVA resulted in the generation of systemic tolerance upon re-challenge (Fig. 2C; $p = 0.007$ vs. saline control CCR9^{-/-} mice). Inhibition of CCR9 with CCX507 (30 mg/kg) dosed during the oral antigen challenge phase did not alter the ability of mice to generate immune tolerance to either dose of oral OVA (Fig. 2D and E; $p < 0.05$ vs. saline control). Additional experiments were conducted in which CCX507 (30 mg/kg) was dosed prior to the initial antigen exposure and throughout the remainder of the experiment; the same experimental setup was also tested using 300 mg/kg CCX507. In both cases inhibition of CCR9 did not result in any detectable deficiencies in the ability of these mice to develop normal immune tolerance to orally administered antigen (Fig. 2F and G; $p < 0.05$ vs. saline control).

Genetic deletion of drug targets in mice can result in phenotypes that differ from those obtained with pharmacological blockade of the same target in adult animals. One such example is CCR4, in which the oxazolone-induced allergic inflammation is exacerbated in CCR4^{-/-} mice [11], while pharmacological intervention with a CCR4 antagonist results in significant therapeutic

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