Reply to Othy et al.: Dendritic cell-specific expression of CCR4 is required for development of EAE

We appreciate the comments of Othy et al. (1) in response to our recently published article (2). The authors treated myelin oligodendrocyte glycoprotein (MOG)-immunized mice with CC chemokine receptor 4 (CCR4) antagonists and did not observe an altered course of disease (1). Thus, they give a warning on rapid translational studies. The most important aspect of our study was the finding that dendritic cells (DCs) are the relevant cellular subset mediating CCR4-dependent effects in experimental autoimmune encephalomyelitis (EAE) development (2). This prominent role of CCR4-expressing DCs in EAE was unexpected, because DCs expressed only low levels of CCR4 compared with T-cell subsets. We studied CCR4 expression in several T-cell subsets in the mouse and found elevated levels of CCR4 mRNA expression as expected in T regulatory cells and T helper (Th) 2 cells, as well as in Th17 cells (Fig. 1). In accordance with our findings, human Th17 cells were also shown to express CCR4 (3). This broad expression pattern of CCR4 points to a high level of complexity of CCR4-mediated effects in the pathogenesis of CNS autoimmunity and has to be taken into account in translational studies. However, our findings demonstrate that cell-specific effects of CCR4 control EAE development, and we therefore emphasized the importance of cell-specific pharmacological inhibition of CCR4 instead of using systemic blockade. We would further like to stress the need for a clear distinction between studies conducted on chemokine receptor-deficient mice and those conducted using antagonists, which may lead to conflicting results. For example, CXCR3$^{-/-}$ mice exhibit increased severity in the acute and the chronic phases of MOG-EAE, whereas blocking CXCR3 with synthetic antagonists inhibited EAE development (4). Because of the complex interplay between chemokine receptors and their ligands, these controversial findings cannot be explained easily. For example, chemokine receptors can act as scavenger molecules, and this may lead to elevated levels of chemokines in the absence of their cognate receptors, or after pharmacological blockade (5). Obviously, such effects are constitutive in gene KOs but not in pharmacological studies. On the other hand, many antagonist studies use DMSO as a solvent, which adversely affects the blood–brain barrier and may influence brain infiltration of mononuclear cells (6). We feel that the most pressing issue related to the therapeutic application of CCR4 antagonists is the investigation of cell-specific blockade of chemokine/chemokine interactions in models of CNS autoimmunity.

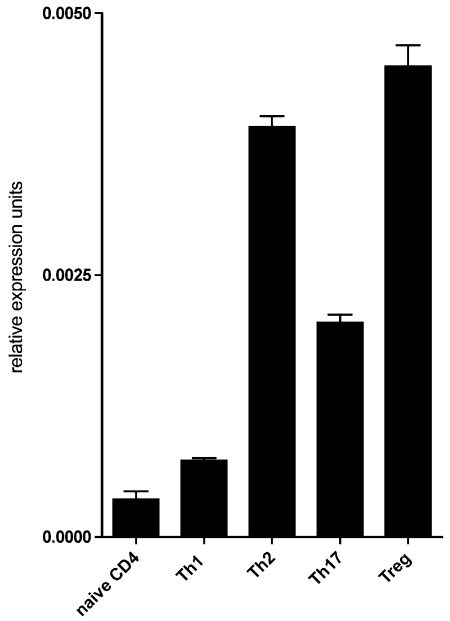

Fig. 1. CCR4 mRNA expression in T-cell subsets. Quantitative RT-PCR assay for CCR4 mRNA in various T-cell subsets differentiated in vitro from C57BL/6 mice. Magnetic bead–sorted splenic CD4$^+$ T cells were stimulated with plate-bound αCD3 antibody (4 μg/mL) or αCD28 antibody (4 μg/mL), together with TGF-β (5 ng/mL) and IL-6 (20 ng/mL) for Th17 differentiation; with IL-12 (10 ng/mL) for Th1 differentiation; with IL-4 (10 ng/mL) for Th2 differentiation; or with TGF-β (5 ng/mL) for regulatory T-cell (Treg) differentiation. A quantitative RT-PCR assay of cDNA samples was performed. The ratio of the average copies of gene mRNA per copy of GAPDH mRNA was determined (mean ± SEM).

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1. Othy S, Topçu S, Kaveri SV, Bayry J (2012) Effect of CC chemokine receptor 4 antagonism on the evolution of experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 109:E2412–E2413.
2. Poppensieker K, et al. (2012) CC chemokine receptor 4 is required for experimental autoimmune encephalomyelitis by regulating GM-CSF and IL-23 production in dendritic cells. *Proc Natl Acad Sci USA* 109:3897–3902.
3. Lim HW, Lee J, Hillsamer P, Kim CH (2008) Human Th17 cells share major trafficking receptors with both polarized effector T cells and FOXP3+ regulatory T cells. *J Immunol* 180(1):122–129.
4. Prendergast CT, Anderton SM (2009) Immune cell entry to central nervous system—Current understanding and prospective therapeutic targets. *Endocr Metab Immune Disord Drug Targets* 9:315–327.
5. Ransohoff RM (2009) Chemokines and chemokine receptors: Standing at the crossroads of immunobiology and neurobiology. *Immunity* 31:711–721.
6. Rosenberg GA, Estrada EY, Mobashery S (2007) Effect of synthetic matrix metalloproteinase inhibitors on lipopolysaccharide-induced blood-brain barrier opening in rodents: Differences in response based on strains and solvents. *Brain Res* 1133(1):186–192.

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