Erschienen in: The EMBO Journal ; 39 (2020). - e105854. - EMBO Press
https://dx.doi.org/10.15252/embj.2020105854

CCR5 deficiency/CCR5Δ32: resistant to HIV infection at the cost of curtailed CD4$^+$ T cell memory responses

Christoph Matti[1] & Daniel F Legler[1,2,3,*]

Orchestrated trafficking and activation by pathogen-derived peptides define the ability of CD4$^+$ T helper cells to contribute to an effective adaptive immunity. In this issue of *The EMBO Journal*, Martín-Leal *et al* show that the inflammatory chemokine receptor CCR5, well known for its role in cell migration and HIV infection, regulates ceramide synthesis and TCR nanoclustering to promote memory CD4$^+$ T cell activation.

The proper function of immune surveillance relies on well-coordinated guidance of patrolling immune cells through peripheral tissues and the homing to secondary lymphoid organs. A tightly regulated network of chemokines and their cognate chemokine receptors orchestrates leukocyte trafficking. Naïve T and B cells recirculate between the blood and secondary lymphoid organs in search for cognate antigens and enter lymph nodes via high endothelial venules in a chemokine receptor CCR7-dependent manner. Upon pathogen encounter in peripheral tissues, dendritic cells (DCs) upregulate CCR7, which guides them to draining lymph nodes where they present pathogen-derived antigens to cognate T cells. Notably, engagement of naïve CD4$^+$ T cells with antigen-presenting DCs triggers the secretion of the chemokines CCL3 and CCL4 resulting in a CCR5-dependent attraction of naïve CD8$^+$ T cells to the DC-CD4$^+$ T cell conjugates, which promotes CD8$^+$ T cell survival and memory generation (Castellino *et al*, 2006). The same DC-derived chemokines were found to recruit CCR5 on CD4$^+$ T cells to the immunological synapse where the chemokine receptor can act as co-stimulatory molecule (Molon *et al*, 2005). CCR5 accounts also for an accelerated recruitment of memory CD8$^+$ T cells to lung airways during respiratory virus infections (Kohlmeier *et al*, 2008).

Besides guiding leukocytes, CCR5 serves as the major HIV co-receptor controlling susceptibility to macrophage-tropic HIV infection and early disease progression in human. Resistance to HIV infection within the human population led to the identification of a 32-base pairs deletion in the *ccr5* gene causing a frameshift and truncation of CCR5. This co-called CCR5Δ32 variant possesses four instead of seven transmembrane domains and is not expressed on the cell surface. Homozygous *ccr5Δ32* individuals are resistant to macrophage-tropic HIV infection and seemingly healthy, suggesting that a CCR5 deficiency is relatively innocuous. However, these individuals are prone to fatal infections, i.e., by West Nile, influenza, or tick-borne encephalitis viruses, which is thought to be due to inefficient leukocyte recruitment (Bachelerie *et al*, 2014).

In this issue of *The EMBO Journal*, Martín-Leal *et al* (2020) describe that CCR5 deficiency in mice leads to an impaired CD4$^+$ T cell memory response. They observed in an OVA-encoding vaccinia virus model that less antigen-specific CD4$^+$ memory T cells produced IFNγ in response to antigenic re-stimulation if mice were deficient for CCR5. Notably, the total number of memory CD4$^+$ T cells and the percentage of effector memory CD4$^+$ T cells are comparable between CCR5-deficient and -proficient mice. Similarly, a prime-boost hapten (NIP-OVA)-mediated, T cell-dependent humoral immune response model revealed no difference in the absolute number or the percentage of memory CD4$^+$ T cells, but CCR5 deficiency led to a markedly reduced generation of high-affinity, class-switched antibodies. These data suggest that CCR5 does not hamper memory development itself, but rather affects the strength of the TCR signal or the TCR's sensitivity to antigens and consequently activation of memory T cells. Indeed, Martín-Leal *et al* (2020) discovered that CCR5 signaling enables the TCR to form nanoclusters on antigen-experienced CD4$^+$ T cells, but not on naïve cells (Fig 1). Electron microscopy analysis clearly revealed a significant increase in number and size of TCR nanoclusters in CCR5-proficient antigen-experienced CD4$^+$ T cells compared to CCR5-deficient cells. Although only a minority of the TCRs forms nanoclusters, these few TCR clusters are sufficient and necessary for a full T cell activation, particularly at low antigen concentrations (Kumar *et al*, 2011; Huang *et al*, 2013). TCR nanoclustering in lymphoblasts strictly relied on CCR5-induced, G protein-dependent signaling and could not

1 Biotechnology Institute Thurgau (BITg), University of Konstanz, Kreuzlingen, Switzerland
2 Faculty of Biology, University of Konstanz, Konstanz, Germany
3 Theodor Kocher Institute, University of Bern, Bern, Switzerland
*Corresponding author. E-mail: daniel.legler@bitg.ch

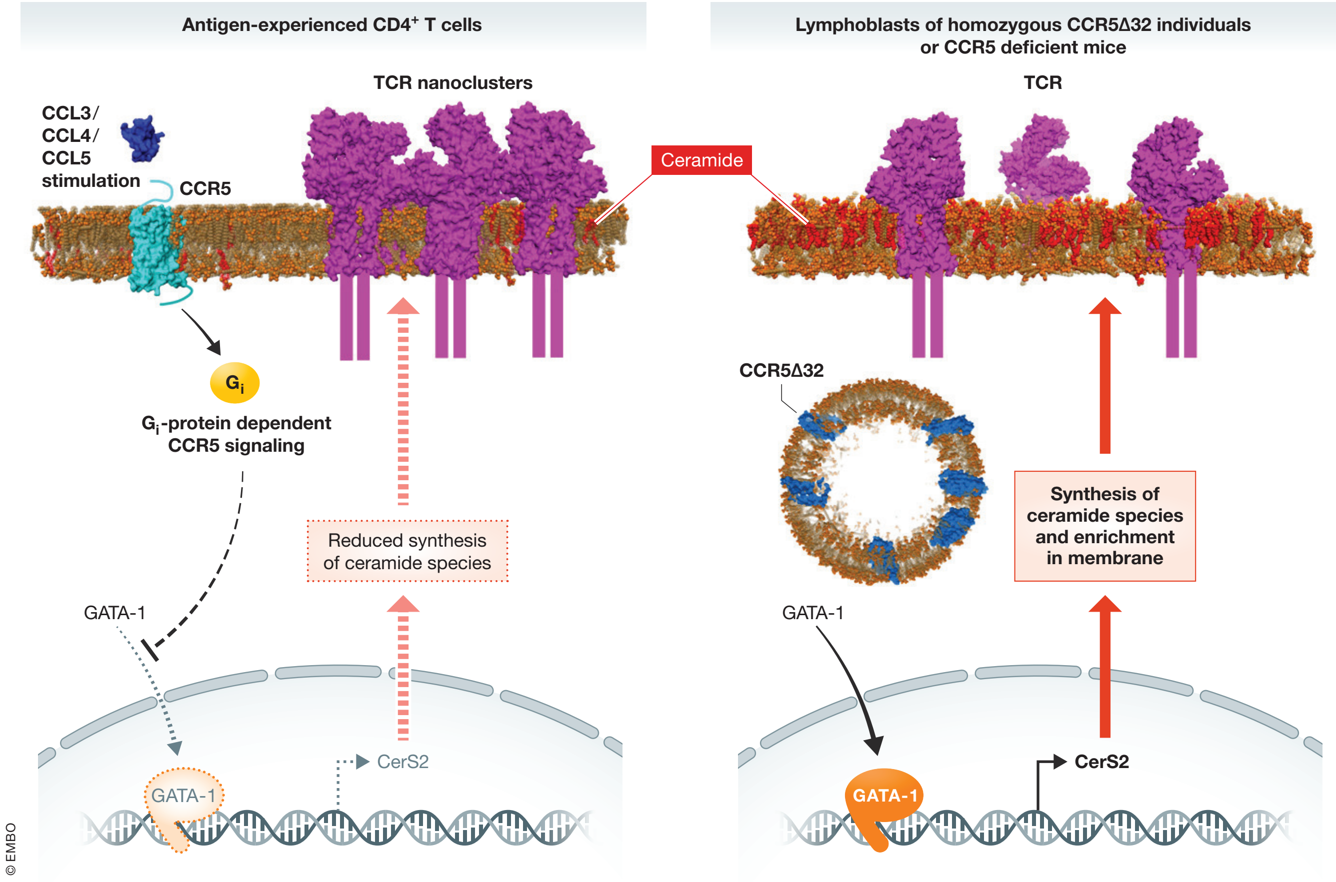

Figure 1. CCR5 signaling dampens ceramide synthesis to enable TCR nanoclustering in antigen-experienced CD4$^+$ T cells.

G_i protein-dependent CCR5 signaling in antigen-experienced, but not naïve CD4$^+$ T cells, correlates with reduced GATA-1-mediated transcription of ceramide synthase 2 (CerS2) and production of ceramide species. Lowering ceramide levels in the plasma membrane of lymphoblasts enables TCR nanoclustering, which in turn increases the antigenic sensitivity of the TCR and ameliorates CD4$^+$ T-cell–B-cell cooperation in humoral responses upon antigen re-encounter. Lymphoblasts of CCR5-deficient mice or homozygous CCR5Δ32 human individuals fail to down-modulate ceramide levels required for TCR nanoclustering.

be attributed to the co-stimulatory activity of CCR5 or to altered TCR expression levels. Interestingly, CCR5-deficient lymphoblasts, but not naïve T cells, showed elevated levels of most ceramide species, including their precursors, while the levels of cellular cholesterol and sphingomyelin species remained unchanged. Particularly, ceramides with longer and saturated acyl chains were enriched in CCR5-deficient lymphoblasts. These ceramide species render the lipid bilayer of the plasma membrane stiffer and are therefore likely to interfere with TCR cluster formation. Notably, chemokine receptor clustering or oligomerization, promoted by modulated cholesterol levels, was found to enable the integration of distinct signaling pathways of CCR7 required for efficient DC and effector T cell migration (Hauser *et al*, 2016; Legler *et al*, 2017). Martín-Leal *et al* (2020) uncovered that specific ceramide synthases are transcriptionally upregulated in CCR5-deficient lymphoblasts and that ceramide levels control TCR nanoclustering in these cells (Fig 1). An increased nuclear level of the phosphorylated and active form of the transcription factor GATA-1 in CCR5-deficient lymphoblasts suggests that CCR5 signaling reduces ceramide levels in antigen-experienced CD4$^+$ T cells by limiting GATA-1-driven transcription of ceramide synthases. Notably, the formation of TCR nanoclusters was also impaired in lymphoblasts of homozygous *ccr5Δ32* individuals, which is concomitant with enhanced levels of ceramide synthase 2 mRNA and saturated ceramide species. Collectively, this elegant study by Martín-Leal *et al* (2020) reveals that CCR5 signaling barely affects memory CD4$^+$ T cell differentiation, but facilitates TCR nanoclustering and ameliorates the antigenic sensitivity and memory CD4$^+$ T cell activation upon antigen re-encounter. Consequently, homozygous *ccr5Δ32* individuals may benefit from being resistant to HIV infection at the cost of curtailed CD4$^+$ T cell memory and, potentially, T cell-dependent humoral responses. It remains to be investigated whether CCR5 signaling, through modulating ceramide levels and consequently the stiffness of the membrane, also effects chemokine receptor nanoclustering and the chemokine receptor-TCR cross-talk (Schaeuble *et al*, 2011). If so, signaling-induced CCR5 oligomerization may in addition contributes to an enhanced migratory capacity of antigen-experienced T helper cells and hence to a more effective memory immune response.

References

Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, Horuk R, Sparre-Ulrich AH, Locati M, Luster AD *et al* (2014) International union of pharmacology. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. *Pharmacol Rev* 66: 1 – 79

Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C, Germain RN (2006) Chemokines enhance immunity by guiding naive $CD8^+$ T cells to sites of $CD4^+$ T cell-dendritic cell interaction. *Nature* 440: 890 – 895

Hauser MA, Schaeuble K, Kindinger I, Impellizzieri D, Krueger WA, Hauck CR, Boyman O, Legler DF (2016) Inflammation-Induced CCR7 oligomers form scaffolds to integrate distinct signaling pathways for efficient cell migration. *Immunity* 44: 59 – 72

Huang J, Brameshuber M, Zeng X, Xie J, Li QJ, Chien YH, Valitutti S, Davis MM (2013) A single peptide-major histocompatibility complex ligand triggers digital cytokine secretion in CD4 (+) T cells. *Immunity* 39: 846 – 857

Kohlmeier JE, Miller SC, Smith J, Lu B, Gerard C, Cookenham T, Roberts AD, Woodland DL (2008) The chemokine receptor CCR5 plays a key role in the early memory $CD8^+$ T cell response to respiratory virus infections. *Immunity* 29: 101 – 113

Kumar R, Ferez M, Swamy M, Arechaga I, Rejas MT, Valpuesta JM, Schamel WW, Alarcon B, van Santen HM (2011) Increased sensitivity of antigen-experienced T cells through the enrichment of oligomeric T cell receptor complexes. *Immunity* 35: 375 – 387

Legler DF, Matti C, Laufer JM, Jakobs BD, Purvanov V, Uetz-von Allmen E, Thelen M (2017) Modulation of chemokine receptor function by cholesterol: new prospects for pharmacological intervention. *Mol Pharmacol* 91: 331 – 338

Martín-Leal A, Blanco R, Casas J, Sáez ME, Rodríguez-Bovolenta E, de Rojas I, Drechsler C, Real LM, Fabrias G, Ruíz A *et al* (2020) CCR5 deficiency impairs $CD4^+$ T-cell memory responses and antigenic sensitivity through increased ceramide synthesis. *EMBO J* 39: e104749

Molon B, Gri G, Bettella M, Gomez-Mouton C, Lanzavecchia A, Martinez AC, Manes S, Viola A (2005) T cell costimulation by chemokine receptors. *Nat Immunol* 6: 465 – 471

Schaeuble K, Hauser MA, Singer E, Groettrup M, Legler DF (2011) Cross-talk between TCR and CCR7 signaling sets a temporal threshold for enhanced T lymphocyte migration. *J Immunol* 187: 5645 – 5652