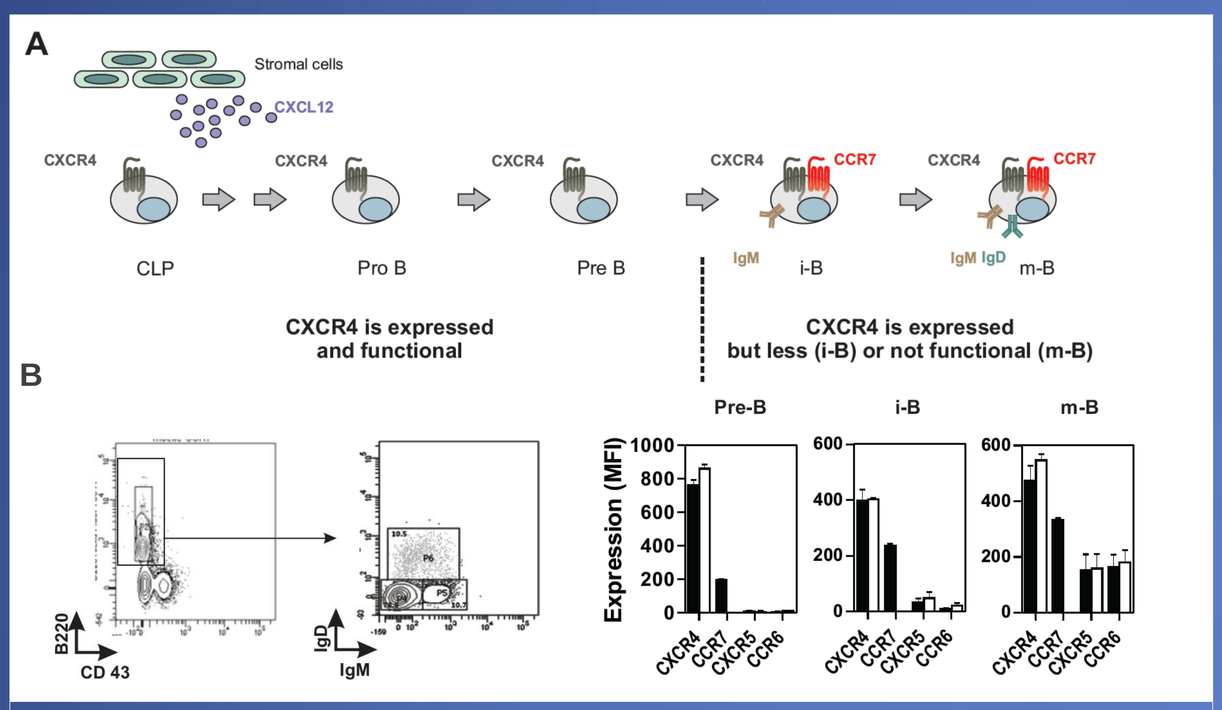
Chemokine receptor CCR7 controls CXCR4 responsiveness during B cells development

Saria Mcheik[1], Nils Van Eeckhout[1], Cédric De Poorter[1], Céline Galès[2], Marc Parmentier[1] and Jean-Yves Springael[1]

[1]Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM) Université Libre de Bruxelles (U.L.B.), Campus Erasme, 808 Route de Lennik, B-1070 Brussels, Belgium.
[2]Institut des Maladies Métaboliques et Cardiovasculaires, Institut National de la Santé et de la Recherche Médicale, Université Toulouse III Paul Sabatier, Toulouse, France.

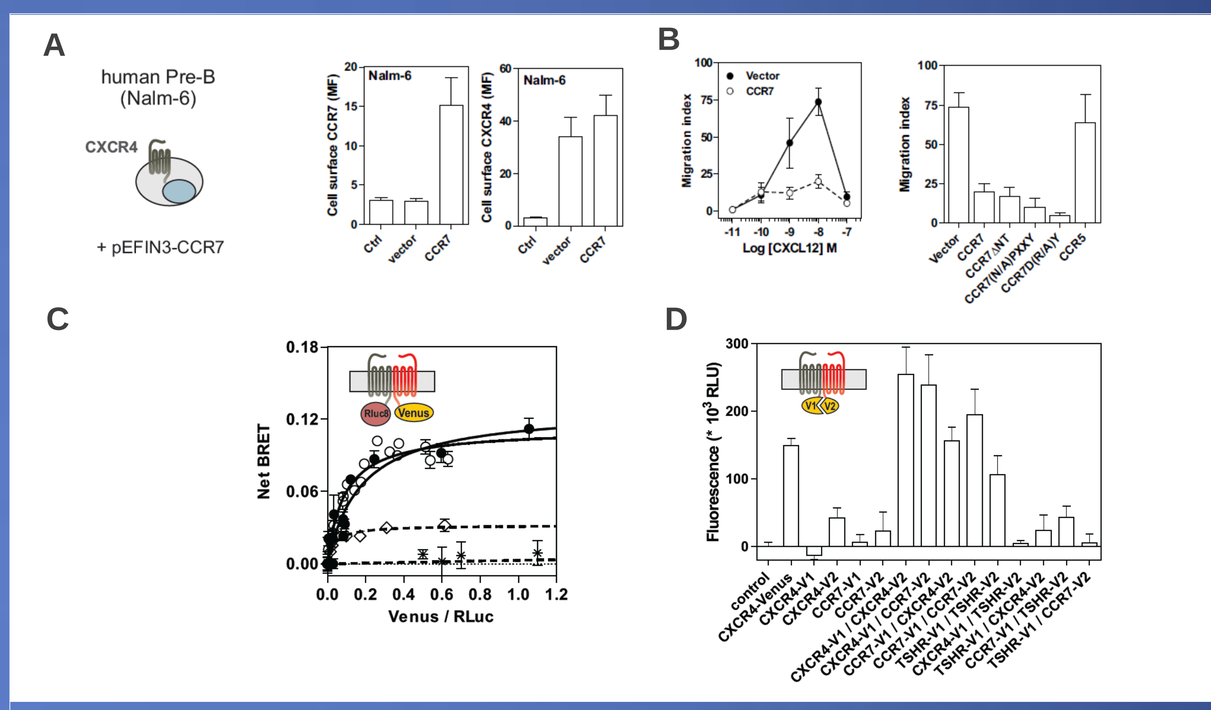
B cells lymphopoiesis in bone marrow is a complex process that requires the precise orchestration of cellular signals like the CXCL12-CXCR4 axis that plays a major role in the retention of stem cells and progenitors in dedicated bone marrow niches. It has been known for more than a decade that CXCR4 responsiveness decreases dramatically after the differentiation of Pre B cells to immature and mature B cells in bone marrow. However, the molecular mechanism underlying this regulation and whether it plays a role in B cells homeostasis remain yet unknown. In this study, we showed that last stages of B cells development in bone marrow go through important physiological changes that modify the cell surface expression of chemokine receptors i.e. the expression of CXCR4 decreases about two-fold while the expression of CCR7 and other receptors is on the increase. We showed that CCR7-deficiency is sufficient to alleviate the inhibition of CXCR4 responsiveness in B cells and to improve their homing in bone marrow. We also provided evidences that this regulation does not require CCR7 signaling nor the scavenging of G proteins by CCR7. We proposed and discussed a model in which the upregulation of CCR7 stabilizes distinctive CXCR4/G protein complex conformation, likely through receptor heteromerization, possessing a reduced capacity to activate Gαi proteins. The discovery that CXCR4 responsiveness during B cells development depends of its oligomerization status constitutes the first indication of a role of chemokine receptor heteromerization in a physiological process.

Chemokine receptors expression during B cells development



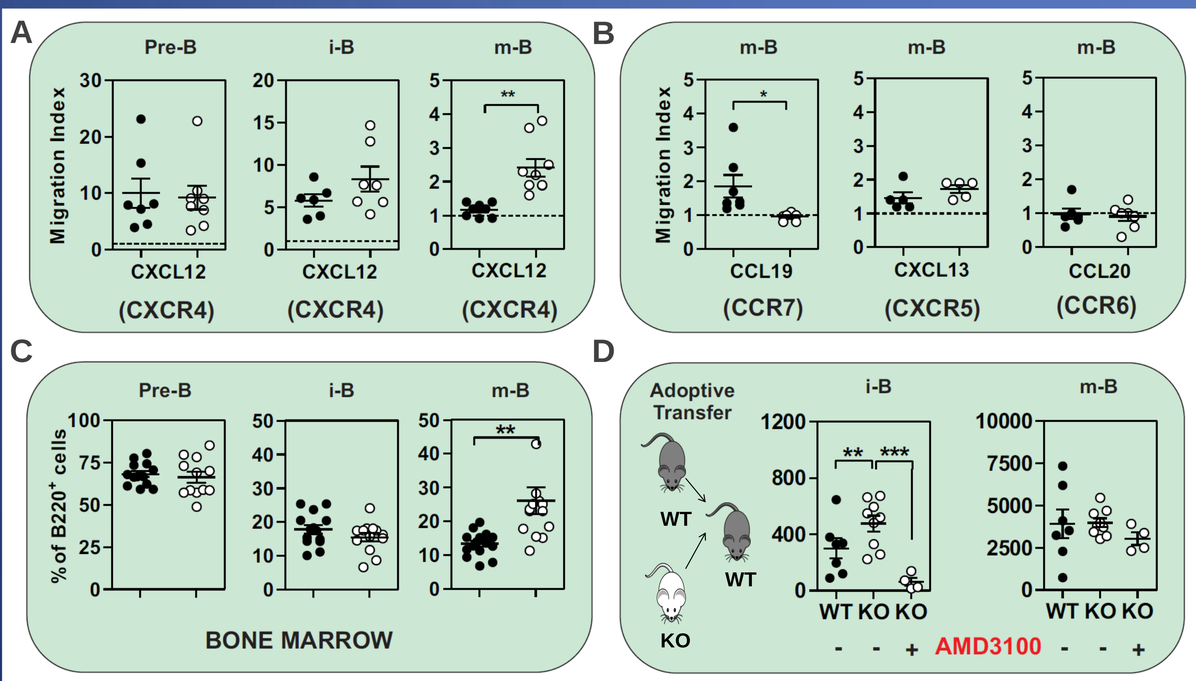
A) B cells lymphopoiesis. A Framework for delineating late stages in development of B lineage cells in mouse bone marrow, based on changes in cell-surface molecule. Abbreviations: CLP: common lymphoid progenitor, i-B: immature B cells, m-B: mature B cells. Of importance, it has been known for more than a decade that CXCR4 responsiveness decreases dramatically after the differentiation of Pre B cells to immature and mature B cells. **B) Expression of chemokine receptors during B cells development.** Bone marrow B cells were discriminated into three subpopulations according to the cell surface expression of four markers: [1] Pre- ($B220^+/CD43^-/IgM^-/IgD^-$); [2] immature ($B220^+/CD43^-/IgM^+/IgD^-$) and [3] mature ($B220^+/CD43^-/IgM^+/IgD^+$) B cells and the expression level of CXCR4, CCR7, CXCR5 and CCR6 was estimated in $CCR7^{-/-}$ mice (white bars) and WT siblings (black bars) using specific antibodies.

CCR7 expression is sufficient to inhibit CXCR4 responsiveness



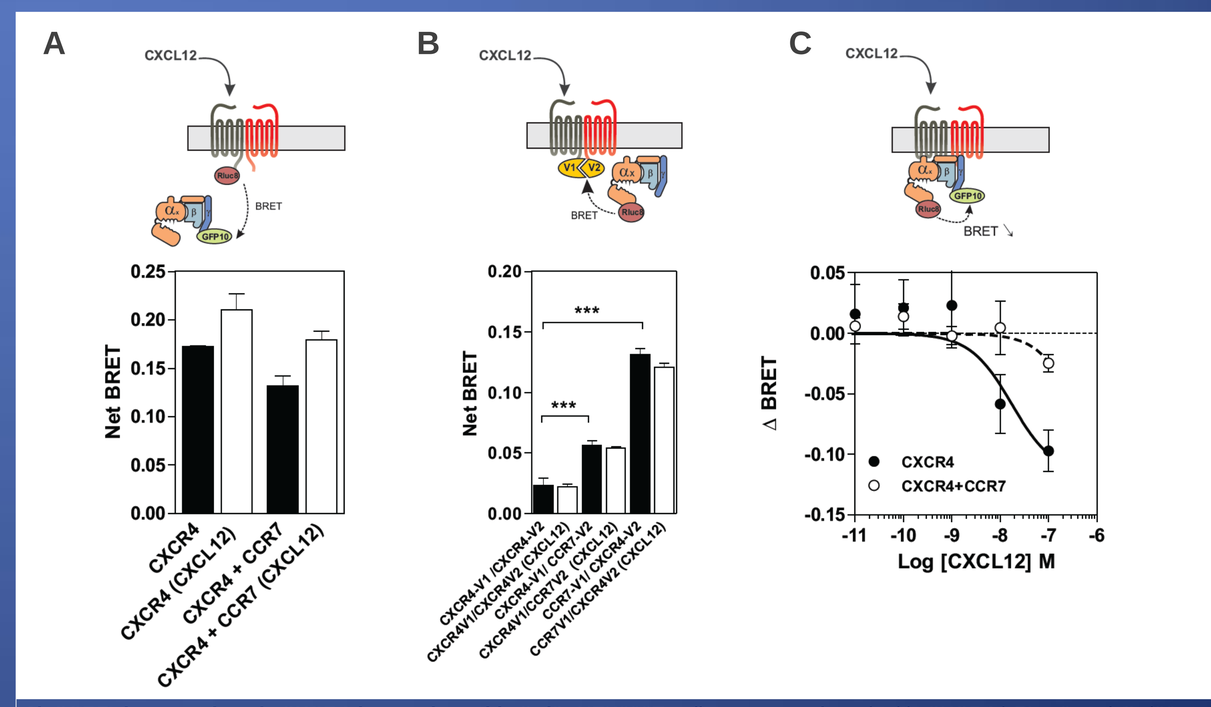
A) Expression of CCR7 in Nalm-6 cells inhibits CXCR4 responsiveness. Nalm-6 cells stably expressing CCR7 (●) or mock-transfected (●) were exposed to CXCL12 before estimation of the migration index. **B) Expression of non-functional CCR7 mutants in Nalm-6 cells inhibits CXCR4 responsiveness.** Nalm-6 cells expressing wild type or non-functional CCR7 mutants were exposed to 1 nM CXCL12 before estimation of the migration index. **C**) **CCR7/CXCR4 heteromerization as measured by BRET** HEK293T cells were transfected with a constant amount of CCR7-h*R*Luc and increasing amounts of the CXCR4-Venus (●), or a constant amount of CXCR4-h*R*Luc and increasing amounts of the CCR7-Venus (●). As a control, TSHR-Venus was used as BRET acceptor with CXCR4-h*R*Luc (✳) or CCR7-h*R*Luc (◆). **D) CCR7/CXCR4 heteromerization as measured by Venus complementation.** HEK 293T cells were transfected with CXCR4-V1, CXCR4-V2, CCR7-V1 and CCR7-V2 only or in a combination, and the fluorescence was recorded. As controls, TSHR-V1 and TSHR-V2 were cotransfected with the CXCR4 and CCR7 constructs.

CCR7 controls CXCR4 responsiveness and B cells homing



A) CCR7-deficiency alleviates the inhibition of CXCR4 responsiveness in B cells. Bone marrow mononuclear cells from $CCR7^{-/-}$ mice (●) and WT siblings (●) were subjected to migration towards a gradient of CXCL12 and migrating cell populations were discriminated by flow cytometry. **B) CCR7-deficiency does not impact on CXCR5 and CCR6 responsiveness.** Bone marrow mononuclear cells from $CCR7^{-/-}$ mice (●) and WT siblings (●) were subjected to migration towards a gradient of CCL19, CXCL13 or CCL20. **C) Mature B cells accumulate in bone marrow of $CCR7^{-/-}$ mice**. Bone marrow mononuclear B cells from $CCR7^{-/-}$ mice (●) and WT siblings (●) were discriminated by flow cytometry and quantified using counting beads. **D) CCR7-deficiency increases the homing of immature B cells in bone marrow.** Bone marrow $B220^+$ cells from $CCR7^{-/-}$ mice (●) and WT siblings (●) were fluorescently labeled and transferred into wild type mice treated or not with AMD3100. Two hours after adoptive transfer, the bone marrow cells were purified and analyzed by flow cytometry

CCR7 alters the conformation and activation of CXCR4-Gαi2



A) CCR7 does not impair CXCR4 interaction with Gαi2. HEK293T cells were transfected with CXCR4-h*R*Luc8 and Gαi2βγ-GFP10 in combination or not with CCR7 and interaction of Gαi2βγ with CXCR4 was investigated by measuring the energy transfer between the two partners **B) Receptor-Gαi2 complex conformation is different in CXCR4/CCR7 heteromer.** HEK293T cells were transfected with h*R*Luc8-Gαi2βγ and (CXCR4-V1 + CXCR4-V2), (CXCR4-V1 + CCR7-V2) or (CXCR4-V2 +CCR7-V1). The interaction of Gαi2βγ with CXCR4/CXCR4 or CXCR4/CCR7 was investigated by measuring the energy transfer between the h*R*Luc8-Gαi2βγ and the reconstituted Venus. **C) CCR7 inhibits the CXCL12-induced activation of Gαi2.** Real-time measurement of BRET signal in HEK293T cells coexpressing the Gαi2 biosensor and CXCR4 only (●) or CXCR4 + CCR7 (●) Cells were stimulated one minute with increasing concentrations of CXCL12 after addition of coelenterazine 400. Results are expressed as ΔBRET corresponding to the difference in BRET signal measured in the presence and absence of CXCL12