The CCL19/CCL21 axis regulates Memory B cell appearance and migration

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DECIDE Project No. 315902

Introduction

ACKR4, previously known CCRL1, is an atypical chemokine receptor (Ulvmar, 2011) and as so, is able to internalise its ligands (CCL19, CCL21 and CCL25) without causing a signalling cascade but regulating their availability and creating gradients in serum and lymph nodes (Comeford, 2006). ACKR4 competes with CCR7 for the ligands.

ACKR4 is expressed in the subcapsular sinus endothelium, creating a gradient of CCL21 that guides DCs from the sinus towards the T zone (Ulvmar, 2014).

ACKR4 is differentially expressed in the dark zone of the GC, while CCR7 is expressed in the light zone (Victora,2012).

We show here that this differential expression has effects in the generation of memory B cells (MBCs) and in their migration to other sites. As CCR7 expression remains constant through all B cells populations, our work is focused on ACKR4, whose expression regulates the response of CCR7-expressing cells to CCL19/CCL21.

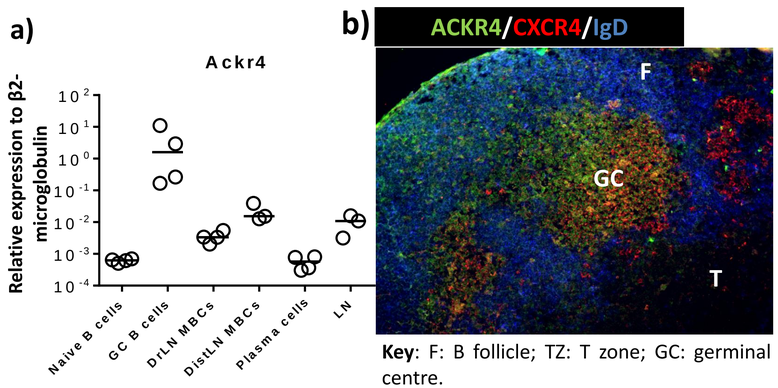
Aim

Study the effect of Atypical Chemokine receptor 4 (ACKR4) deficiency in B cells and in the environment in MBC generation and migration to distant lymphoid sites.

1. ACKR4 expression

ACKR4 is expressed in GC cells at the mRNA and protein level. In non-immunised mice, (which lack GCs) ACKR4 protein is not detectable in any haematopoietic cells, but is expressed in the subcapsular sinus of the lymph nodes (Ulvmar, 2014) .

Figure 1. a) Ackr4 expression by qPCR in different B cell subsets. WT mice were transferred with Cγ1-Cre $K^{-/-}$QMmTmG $NP^{+}B220^{+}$ cells and immunised with NP-CGG subcutaneously in footpads. B cells different populations of GFP^{+} cells of were sorted out from drLN and distant LN at day 8 after immunisation. b) Immunofluorescence staining image of a WT popliteal lymph node 8 days after immunisation with 20µg NP-CGG alum precipitated in the footpad.



Key: F: B follicle; TZ: T zone; GC: germinal centre.

Conclusions

- ACKR4 is upregulated at the mRNA and protein levels in GC B cells. ACKR4 is also expressed in the subcapsular sinus of non-reactive lymph nodes. It is also expressed at intermediate levels in MBCs
- ACKR4-deficiency increases the frequency of MBCs appearing in distant LN. This is due to the deficiency of ACKR4 in the B cells themselves and not in the environment.
- There are several steps were ACKR4 can influence MBC migration towards distant sites. The exit from the reactive LN towards the subcapsular sinus is increased when MBCs are $ACKR4^{-/-}$. This explains the higher appearance in remote sites.
- ACKR4 expression in MBCs counteracts CCR7 effect and allows MBCs to traffic toward the sinus away from CCL19 and CCL21.

2. ACKR4-deficiency increases MBC appearance in distant LN

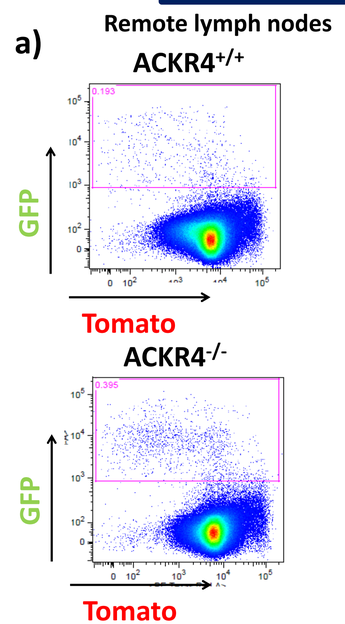
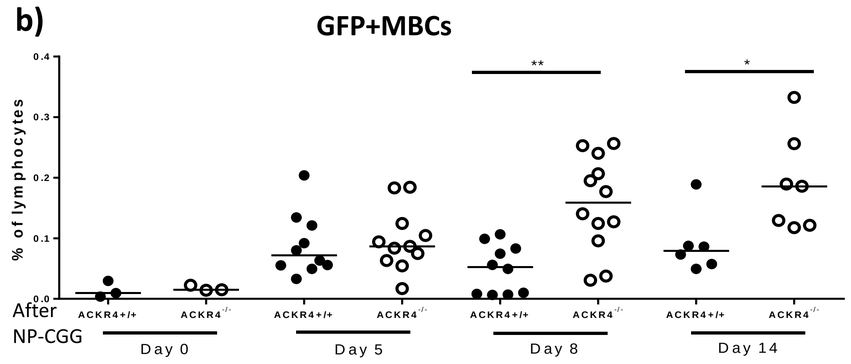


Figure 2. a) Representative flow cytometric graphs of pooled non-reactive LN from $Cγ1^{Cre/wt}$ $κ^{-/wt}$ mTmG$^{+/wt}$ $ACKR4^{+/+}$ and $Cγ1^{Cre/wt}$ $κ^{-/wt}$ mTmG$^{+/wt}$ $ACKR4^{-/-}$ mice 8 days after immunisation with 20µg NP-CGG alum precipitated in the footpad. b) MBC ($GFP^{+}CD38^{+}CD138^{-}$) flow cytometric data from pooled non-reactive LN from $Cγ1^{Cre/wt}$ $κ^{-/wt}$ mTmG$^{+/wt}$ $ACKR4^{+/+}$ and $Cγ1^{Cre/wt}$ $κ^{-/wt}$ mTmG$^{+/wt}$ $ACKR4^{-/-}$ mice at day 0, day 5, day 8 and day 14 after immunisation with 20µg NP-CGG alum precipitated in the footpad. 2-3 independent experiments are pooled together. *p<0.05, **p<0.01



3. ACKR4-deficiency in B cells and in the environment

In order to distinguish if the effect seen in the previous section was caused by the expression of ACKR4 in the B cells or in the environment, ACKR4 sufficient 4-hydroxynitrophenyl-specific (NP) **mTmG**$^{+}$ B cells (QM) and ACKR4 deficient 4-hydroxynitrophenyl-specific (NP) **eYFP**$^{+}$ B cells (QM) are transferred into ACKR4 sufficient and deficient hosts.

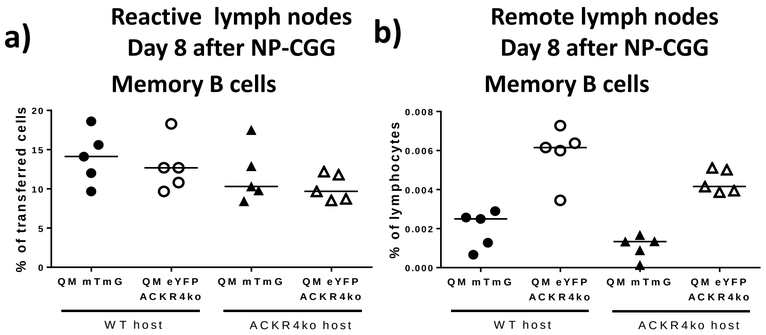


Figure 3. a) MBC (tomato^{+} or eYFP^{+},$CD38^{+}CD138^{-}$) flow cytometric data from popliteal lymph nodes of WT and $ACKR4^{-/-}$ mice transferred with $1x10^{5}$ $NP^{+}B220^{+}$ cells from QM mTmG mouse and $1x10^{5}$ $NP^{+}B220^{+}$ cells from QM eYFP $ACKR4^{-/-}$ mouse, at day 8 after immunisation with 20µg NP-CGG alum precipitated. b) MBC (tomato^{+} or eYFP^{+},$CD38^{+}CD138^{-}$) flow cytometric data from pooled axilary lymph nodes of same mice than in a).

References

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Acknowledgements

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n°315902.