Atypical chemokine receptor 4, ACKR4, in B cell activation

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Introduction

Atypical chemokine receptors have been recently described as regulators of chemokine signalling. ACKR4, or previously known CCRL1, is one of the four components of this family (Ulvmar, 2011).

ACKR4 is able to internalise its ligands (CCL19, CCL21 and CCL25. CXCL13 has only been confirmed in human ACKR4), regulating their availability outside the cells (Comeford, 2006).

ACKR4 is expressed in heart, lung, small intestine, brain, testes and lymph nodes. In the lymph nodes ACKR4 is expressed in the subcapsular sinus, creating a gradient of CCL19, influencing the movement of DCs towards the T zone (Ulvmar, 2014).

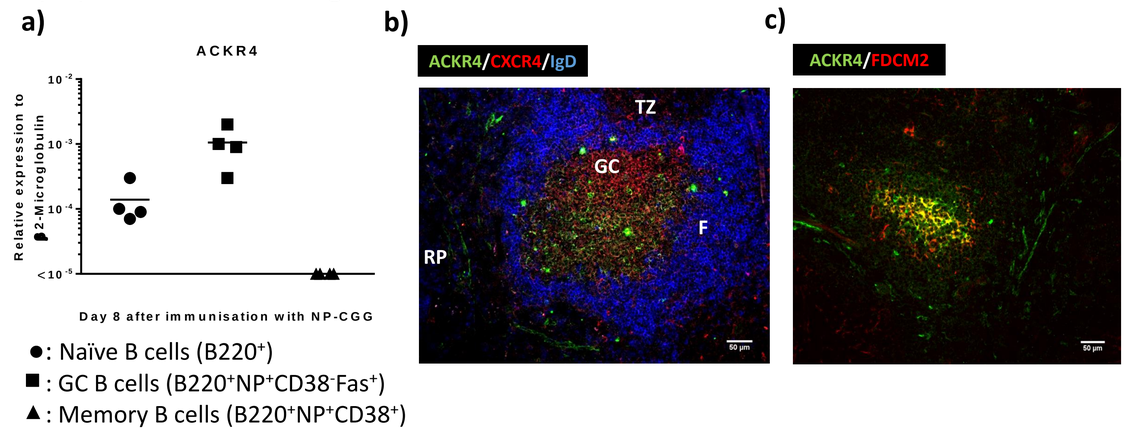
ACKR4 deficient mice present higher incidence of autoimmune diseases such a autoimmune encephalomyelitis (Comerford, 2010). The expression of ACKR4 has been described as a negative marker for metastases in multiple cancer models (breast, colorectal and squamous cell carcinoma).

Aim

Determine the role of the atypical chemokine receptor ACKR4 in the secondary lymphoid organs after TD immunisation and therein in the GC response.

Expression

ACKR4 is expressed in GC B cells at mRNA and protein level, both in B cells and in FDCs.
In non-immunised mice, ACKR4 is not expressed in any haematopoietic cells, but is expressed in the subcapsular sinus of the lymph nodes (Ulvmar, 2014) and in the splenic red pulp stroma surrounding the follicles.



●: Naïve B cells (B220$^+$)
■: GC B cells (B220$^+$NP$^+$CD38$^-$Fas$^+$)
▲: Memory B cells (B220$^+$NP$^+$CD38$^+$)

Figure 1. a) qPCR data of ACKR4 expression in different B cell subsets obtained by sorting from lymph nodes of WT mice transferred with Cγ1-Cre x QM ROSAeYFP B cells 8 days after immunisation with NP-CGG in the footpads. b) and c) Representative immunofluorescence staining images of a WT spleen 8 days after immunisation with SRBC. b) ACKR4 expression in GC B cells. c) ACKR4 expression in FDCs.
Key. F: B follicle; TZ: T zone; RP: red pulp. GC: germinal centre

Conclusions

- The deficiency of ACKR4 is not influencing the B cell response (Germinal centre B cell numbers, plasma cell numbers and antibody titres) at the peak of the Germinal Centre.
- However, the expression of ACKR4 in non-B cells influences GC shape, diminishing the area free of naïve B cells within the Germinal centre area and the distribution of antigen-specific B cells..
- Although this shape modification is not affecting GC output in the peak of the GC response, it could be having an effect at later stages such as maintenance and disappearance of the GC.

ACKR4^{het} and ACKR4^{ko} response to TD antigen

Primary as well as secondary immunization of ACKR4 ko mice with protein antigens initially results in normal plasma cell and GC B cell numbers. Also, there are no differences in antibody titres measured by ELISA. However, ACKR4^{ko} mice show GCs with abnormal shape, with more naïve B cells entering the GC periphery.

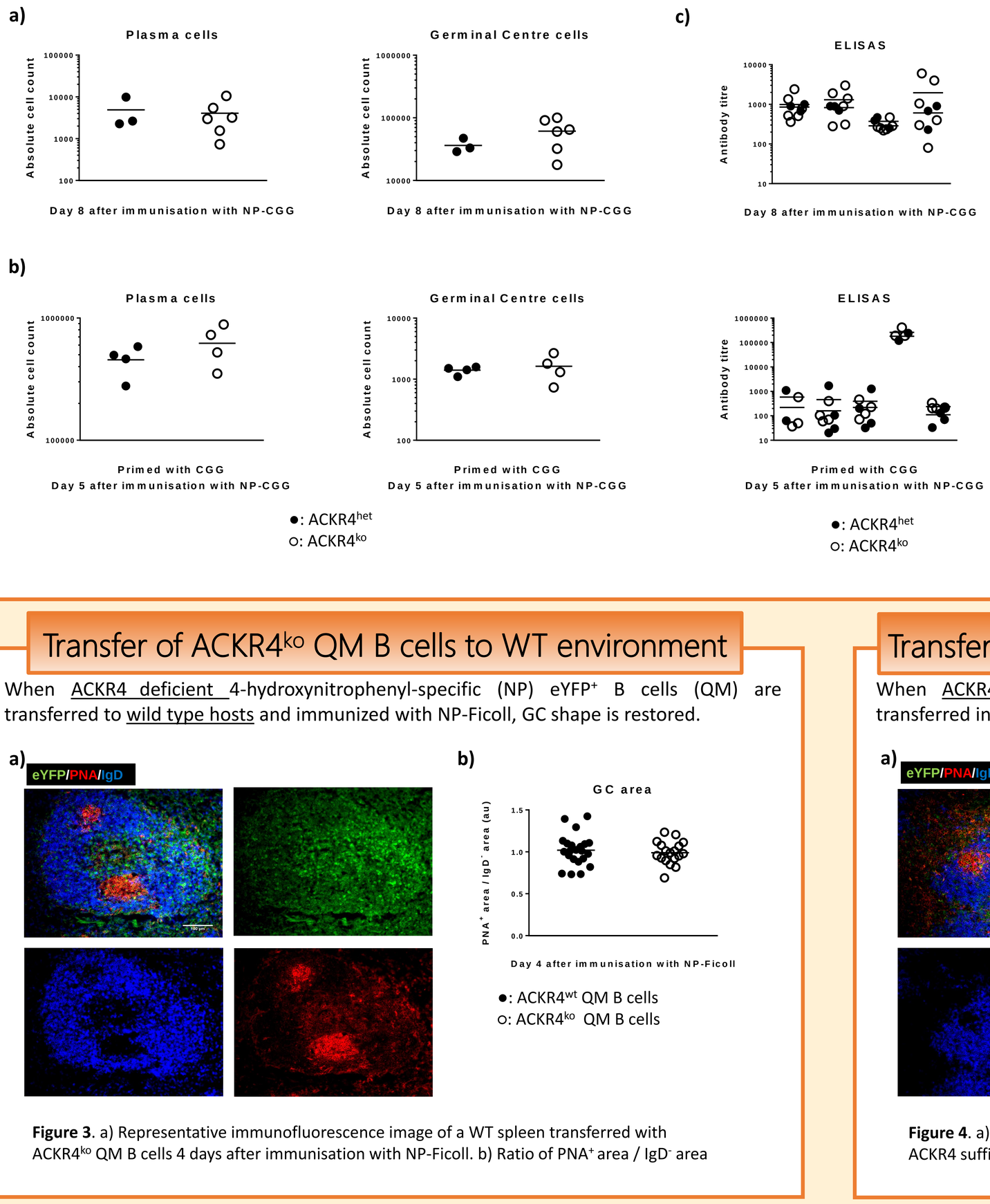


Figure 2. a) Splenic flow cytometry data of plasma cells (B220$^+$NP$^+$CD138$^+$) (left) and GC B cells (B220$^+$NP$^+$CD38$^-$Fas$^+$) (right) from ACKR4^{het} and ACKR4^{ko} mice at day 8 after immunisation with NP-CGG. b) Splenic flow cytometry data of plasma cells (left) and GC B cells (right) from ACKR4^{het} and ACKR4^{ko} mice at day 5 after boost immunisation with NP-CGG. c) Relative antibody titres from ACKR4-^{het} and ACKR4-^{ko} mice immunised like in a) (upper) and like in b) (lower). d) Representative immunohistochemistry staining images of spleens from ACKR4^{het} mice (left) and ACKR4^{ko} mice (right) immunised like a).
Key. F: B follicle; TZ: T zone; RP: red pulp

Transfer of ACKR4^{ko} QM B cells to WT environment

When ACKR4 deficient 4-hydroxynitrophenyl-specific (NP) eYFP$^+$ B cells (QM) are transferred to wild type hosts and immunized with NP-Ficoll, GC shape is restored.

Figure 3. a) Representative immunofluorescence image of a WT spleen transferred with ACKR4^{ko} QM B cells 4 days after immunisation with NP-Ficoll. b) Ratio of PNA$^+$ area / IgD$^-$ area

Transfer of ACKR4^{wt} QM B cells to ACKR4^{ko} environment

When ACKR4 sufficient 4-hydroxynitrophenyl-specific (NP) eYFP$^+$ B cells (QM) are transferred into ACKR4 deficient hosts, GC shape is disturbed again.

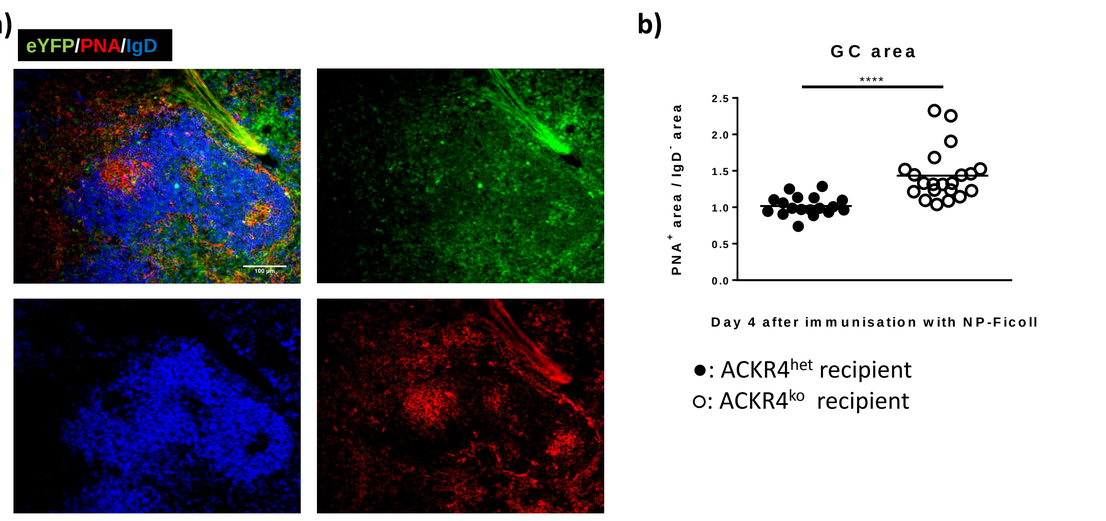


Figure 4. a) Representative immunofluorescence image of a ACKR4 deficient spleen transferred with ACKR4 sufficient QM B cells 4 days after immunisation with NP-Ficoll. b) Ratio of PNA$^+$ area / IgD$^-$ area.

References

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