ACKR4 deficiency leads to Germinal Centres with deregulated shape

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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 for human ACKR4), regulating their availability to 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).

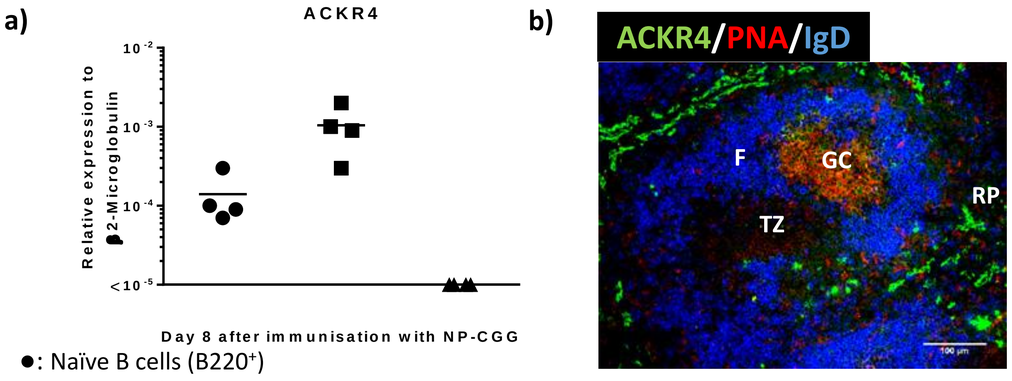
ACKR4 deficient mice have a higher incidence of autoimmune diseases such a autoimmune encephalomyelitis (Comerford, 2010). The expression of ACKR4 has been described as a negative marker for metastasis in multiple cancer models (breast, colorectal and squamous cell carcinoma).

Aim

To determine the role of the atypical chemokine receptor ACKR4 in the secondary lymphoid organs during T cell dependent (TD) antibody responses and in the germinal centre (GC).

1. 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) 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 subcutaneous foot immunisation with NP-CGG. b) Representative immunofluorescence staining image of a WT spleen 8 days after immunisation with SRBC i.v. **Key**: F: B follicle; TZ: T zone; RP: red pulp. GC: germinal centre. Scale bar: 100 µm.

Conclusions

- Deficiency of ACKR4 does not influence the size of the B cell response (GC B cell numbers, plasma cell numbers and antibody titres) at the peak of the GC response.
- However, the expression of ACKR4 in non-B cells influences GC shape, leading to intermingling of IgD^+ naïve B cells and IgD^- GC B cells at the edge of the GC area.
- Although this shape modification does not seem to affect GC output in the peak of the GC response, it may have an effect at later stages, regulating DZ/LZ distribution, maintenance and termination of the GC response.

2. Peak of the GC response

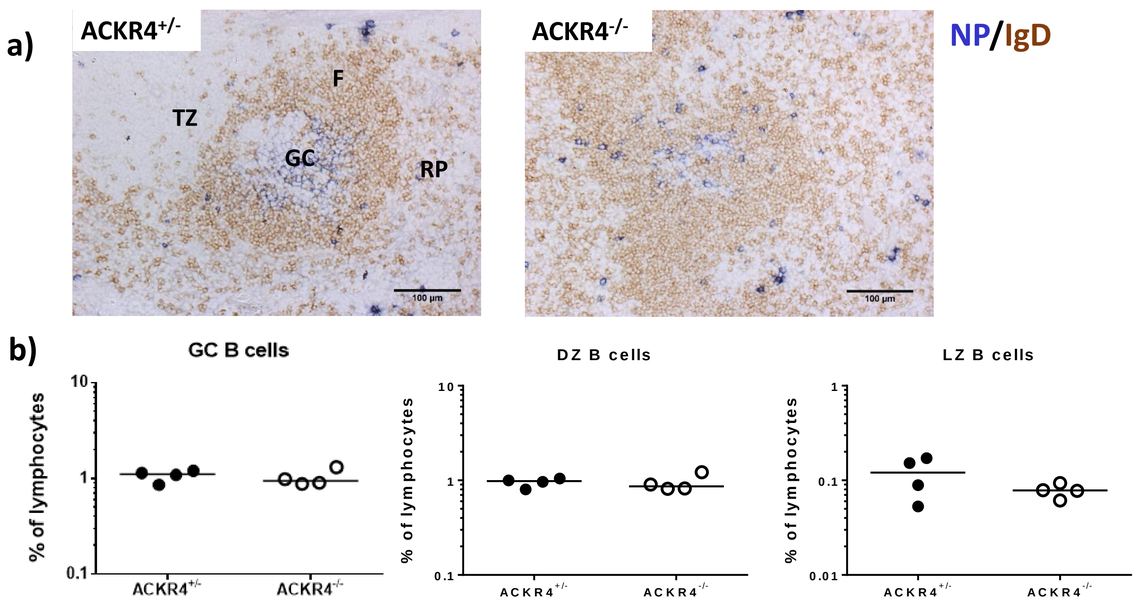


Figure 2. a) Representative immunohistochemistry staining images of spleens from $ACKR4^{+/-}$ (left) and $ACKR4^{-/-}$ mice (right) 8 days after immunisation with NP-CGG in alum i.p. b) Splenic flow cytometry data from $ACKR4^{+/-}$ and $ACKR4^{-/-}$ mice at days 8 after SRBC immunisation i.v. of GC B cells ($B220^+CD38^-Fas^+$) (left), dark zone GC B cells ($B220^+NP^+CD38^-Fas^+CXCR4^+CD86^-$) (middle) and light zone GC B cells ($B220^+NP^+CD38^-Fas^+CXCR4^-CD86^+$) (right). **Key:** F: B follicle; TZ: T zone; RP: red pulp; GC: germinal centre. Scale bar: 100 µm

3. Later stages of the GC response

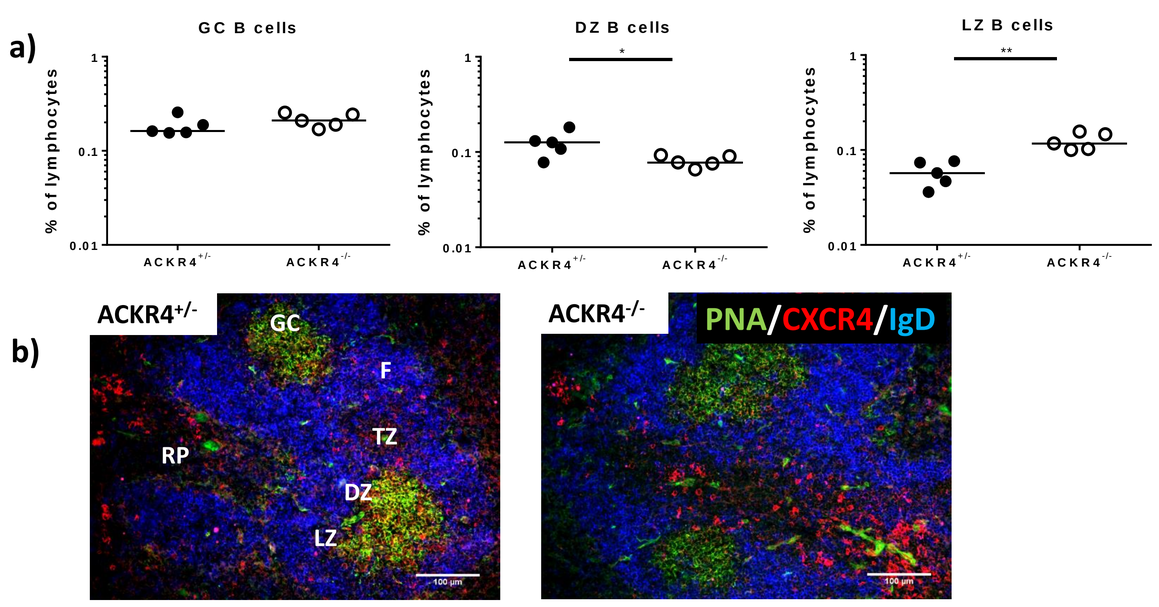


Figure 3. a) Splenic flow cytometry data from $ACKR4^{+/-}$ and $ACKR4^{-/-}$ mice at days 14 after SRBC immunisation of GC B cells ($B220^+CD38^-Fas^+$) (left), dark zone GC B cells ($B220^+NP^+CD38^-Fas^+CXCR4^+CD86^-$) (middle) and light zone GC B cells ($B220^+NP^+CD38^-Fas^+CXCR4^-CD86^+$) (right) b) Representative example of immunofluorescence staining for PNA, CXCR4 and IgD from $ACKR4^{+/-}$ (left) and $ACKR4^{-/-}$ spleens (right) 14 days after SRBC immunisation i.v.
Key: F: B follicle; TZ: T zone; RP: red pulp; GC: germinal centre; LZ: light zone; DZ: dark zone. Scale bar: 100 µm

4. ACKR4-deficiency in B cells and in the 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 normal.

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

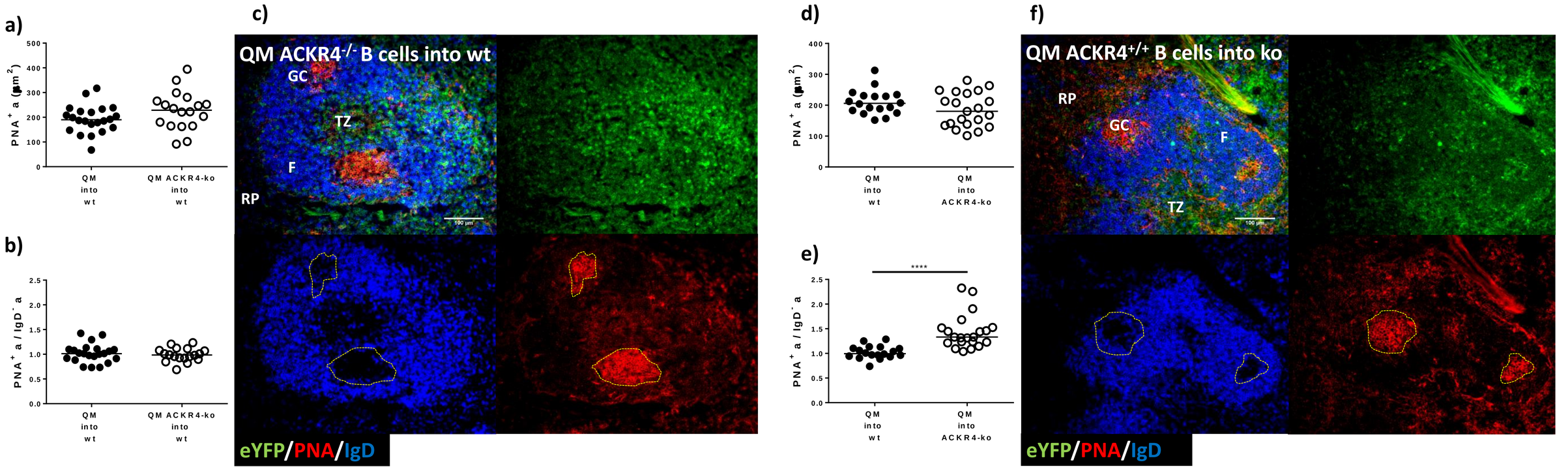


Figure 4. a,b,c: Transfer of $eYFP^+$ QM B cells sufficient and deficient for ACKR4 into a wt environment. a) GC area. b) Fraction of GC infiltrated by naïve B cells. c) Representative example of immunofluorescence staining for eYFP, PNA and IgD in this conditions.

Figure 4. d,e,f: Transfer of $eYFP^+$ QM B cells into a wt or $ACKR4^{-/-}$ environment. d) GC centre area. e) Fraction of GC infiltrated by naïve B cells. f) Representative example of immunofluorescence staining for eYFP, PNA and IgD in this conditions. 18-23 GCs were counted from n=4. **Key:** TZ: T zone; F: B follicle; GC: germinal centre; RP: red pulp. Scale bar: 100 µm

References

Bajoghli, B. (2013). Eur J Immunol 43(7): 1686-1692

Chew, AL. (2013). Biomed Rep 1(2): 185-192

Comerford, I., et al (2006). Eur J Immunol 36(7): 1904-1916

Ulvmar, M. H., et al (2011). Exp Cell Res 317(5): 556-568

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