Chemokine-based decisions in germinal centres

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DECIDE

Introduction

After infection or vaccination, B cells in the lymph nodes and in the spleen proliferate and give rise to plasma cells (in charge of antibody production for fighting the infection) and memory cells (in charge of remembering the infection to make next encounters more effective). This process takes place in germinal centres (GCs), but plasma cells and memory cells have to leave GC s and be directed to the bone marrow.

Chemokines and their receptors are implicated in the movement of cells of the immune system (Bajoghli, 2013). Chemokine gradients make cells to move in the lymphoid organs. Atypical chemokine receptors have been recently described as regulators of chemokine signalling. ACKR4 is one of the four components of this family (Ulvmar, 2011).

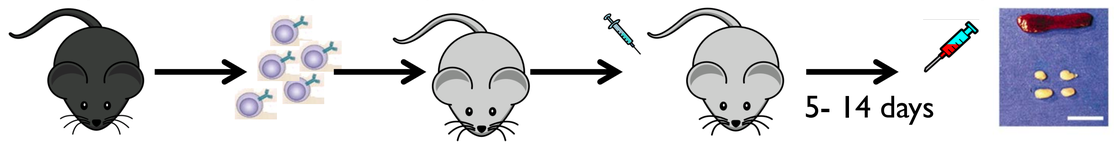
ACKR4 is able to internalise its ligands (CCL19, CCL20 and CCL25 broadly existing in the spleen and lymph nodes), regulating their availability outside the cells (Comeford, 2006). ACKR4 expression in tumours reduces metastasis in colon and breast cancer and it may have a role in regulating immune responses (Chew, 2013).

Aim

Determine the role of the atypical chemokine receptor ACKR4 in the secondary lymphoid organs after vaccination.

Methods

Comparison of wild type mice (WT) and mice deficient in chemokine receptor

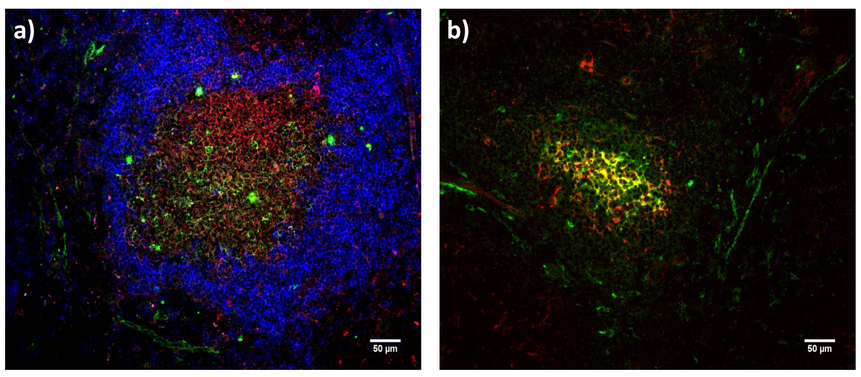


Transfer of B cells deficient for chemokine receptor to WT environment

Results I

ACKR4 is expressed in the Germinal Centres in the murine spleen after vaccination, on both B cells and follicular dendritic cells (FDCs)

Immunofluorescence images of WT spleens 9 days after vaccination a) Stained for Germinal Centre B cells (red), ACKR4 (green) and naïve B cells (blue). b) Stained for FDCs (red) and ACKR4 (green).

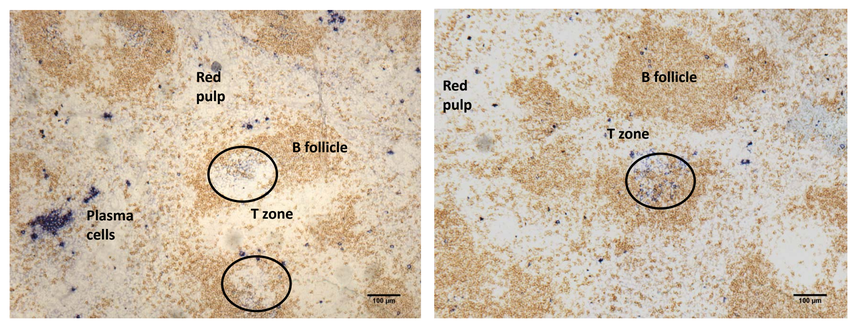


FDCS are in charge of checking if the antibody affinity has increased.

Results II

ACKR4 –deficient mice present Germinal Centres with an abnormal shape

Immunohistochemistry staining of a WT spleen (left) and of a ACKR4-deficient spleen (right) 8 days after vaccination. Germinal centres are marked by black circles with blue cells surrounded by naïve B cells (brown).

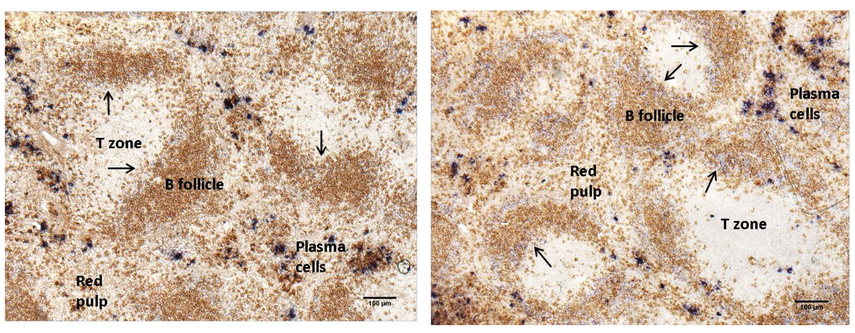


ACKR4-deficient mice present GCs with disorganised structure and boundaries.

Results III

ACKR4 is not influencing the initial movement of cells to the T zone border after vaccination

Immunohistochemistry staining of a WT spleen (left) and of a ACKR4-deficient spleen (right) 4 hours after vaccination.

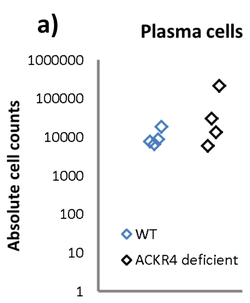


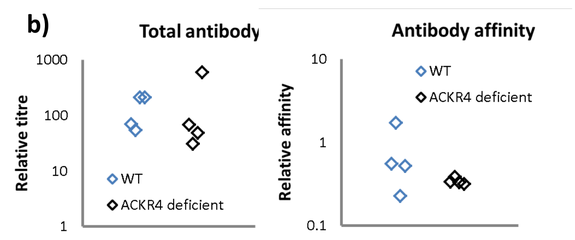
There is no difference in migration of activated cells (light blue, marked with arrows) to the B-T zone border in the absence of ACKR4.

Results IV

ACKR4 does not affect the output of plasma cells after vaccination but it is reducing the affinity of their antibodies

a) Flow cytometry data for plasma cells 5 days after vaccination in a B cell transfer experiment. There are no differences in plasma cell numbers in the absence of ACKR4.





b) Total antibody production (left) is the same for both groups but the affinity (right) is slightly decreased in the mice that were transferred with ACKR4-deficient B cells

Conclusions and future work

ACKR4 is playing a role shaping germinal centres responses and could be implicated in keeping the cells from leaving the germinal centre too soon.
Further characterisation of its role is needed, including how it influences the output of memory cells, the affinity of antibodies. Finally, its role in the migration of both plasma cells and memory cells out of the germinal centres in the spleen and lymph nodes to the bone marrow.
Next experiments will focus on the further description of this system using the mouse models available and new techniques such as intravital microscopy that allow to observe the movement of cells in alive mice.

References

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