ImmunoTools *special* Award 2018

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Towards the understanding of the role of interaction partners of CCR7 in immune cell migration and antigen presentation

Upon an acute or viral infection, dendritic cells (DCs) sense the invading pathogen at epithelial barriers (skin, lungs, mucosal tissue) and transport antigens to the draining lymph nodes to induce a pathogen-specific adaptive immune response. Indeed, upon pathogen encountering DCs take up antigen, start to maturate and induce the expression of co-stimulatory molecules important for T cell priming as well as the chemokine receptor CCR7, recognizing the chemokines CCL19 and CCL21, enabling the cell to migrate to draining lymph nodes. Directional DC migration is achieved by sensing CCL21 gradients produced by lymphatic endothelial cells, which guide DCs from the perilymphatic interstitium into the lymphatic capillaries. Within the lymph flow, DCs are transported into the subcapsular sinus and enter the draining lymph nodes via the afferent lymphatic vessel. They continue their migration into the T cell zone. Similarly, naive circulating lymphocytes ($CD62L^{hi}$ $CD44^{lo}$) enter lymph nodes via high endothelial venules (HEVs) and migrate to the T cell zone via fibroblastic reticular cells (FRCs)-producing CCL21 and scan the surface of DCs for antigen during random migration in the T cell zone. The rare lymphocytes with antigen-specific receptors will proliferate and differentiate into effector cells during the clonal selection and subsequently leave the lymph nodes to fight the infection.

We are currently performing a screening to identify interaction partners of the chemokine receptor CCR7. By using the Hoxb8 progenitor mouse cells and the CRISPR/Cas9 technology, we aim to delete the interaction partner of interest and evaluate its role in immune cell migration and antigen presentation by terminally differentiating the progenitor cells into bone marrow derived dendritic cells (BMDCs). Moreover, a human CCR7 endogenously expressing cell line will be used to confirm our findings in the human system.

Hoxb8 progenitor cell cultures are supplemented with rm Flt3L/CD135 or rm GM-CSF to differentiate the cells into plasmacytoid dendritic cells (pDCs) or inflammatory migrating DCs (cDCs), respectively. Since those populations are heterogeneous, we will need to purify DC subsets using a panel of specific antibodies including CD11c,

CD11b-FITC, CD19-APC, B220 and PDCA. Cells will be further stimulated with LPS or rm TNFa to get matured dendritic cells.

After maturation, CCR7 distribution, signalling and migration will be examined in the presence or absence of rm MIP3b/CCL19 by microscopy. Furthermore, we will evaluate the capacity of the dendritic cells to form an immune synapse with OT-I or OT-II T cells after pulsing them with SIINFEKL or $OVA_{323-339}$ peptide respectively. In a next step, we will investigate whether the identified interaction partners play any role in immune cell migration *in vitro* in 2D and 3D migration assays as well as in a homing assay *in vivo*. Finally, dendritic cells will be pulsed and injected intravenously and the resulting T cell response will be investigated by ELISA for mouse TNFa and by surface markers for activation using CD3-APC, CD4-PE, CD8a-PE, CD25-FITC, CD44-FITC, CD154-FITC, CD134-FITC and CD62L-FITC.

Similar experiments will be done with the human cell line expressing CCR7 after deleting the human homolog of the protein of interest. Indeed, we will also examine the migratory phenotype towards rh MIP3b/CCL19 gradients and the priming capacity of human T cells using surface markers for activation including CD69-APC, CD25-PE as well as the IFN-gamma response by ELISA (human IFN-gamma).