ImmunoTools *special* Award 2014

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mTOR inhibition as a promising strategy for the stabilization of atherosclerotic plaques in mice

Despite recent successes in atherosclerotic plaque stabilization induced by HMG-CoA reductase inhibitors (e.g. Statins), atherosclerosis remains the first killer in the Western world claiming 1 death every 39 seconds in the United States alone. Recent observations have clearly shown that inhibiting the mechanistic target of rapamycin (mTOR) can immensely reduce plaques both in size and in complexity leading to a much more stable form of atherosclerosis in both ApoE deficient mice as well as in LDLR knock-out mice. mTOR inhibitors are currently used in stenting therapy to prevent smooth muscle cell migration and intima thickening. However, the benefit of mTOR inhibitors greatly exceed their ability to prevent smooth muscle cells proliferation and thus a hunt has started to uncover the true mechanisms behind these observations.

In this project we hypothesize that the rapamycin derivative everolimus stabilizes plaques in ApoE deficient mice by one, or a combination of, the following mechanisms: autophagy induction in macrophages, polarization of macrophages to an M2-like status and/or inhibition of intraplaque neovascularization.

To uncover the role of autophagy in the process, we will use $ATG7^{f/f}$ LysM-KI mice which lack the ability to induce autophagy in macrophages.

However, some researchers suggest that suppression of the immune system, and not autophagy induction in macrophages, is the mechanism behind everolimus effects on atherosclerosis. To investigate this, we will treat mice with everolimus and follow immunosuppression. The ImmunoTools anti-mouse antibodies for flow cytometry are a great tool to examine this immunological effect.

Following everolimus treatment by means of a subcutaneously implanted osmotic mini-pump, mice will be euthanized and blood will be collected together with different

organs. To examine the role of everolimus on the circulating immune cells, an immune cell characterization will be performed. The ImmunoTools anti-mouse antibodies for flow cytometry will be put to good use during these experiments. After cleaning the blood sample, we will use a gating strategy developed in our lab to isolate the different cell types. Two tubes for flow cytometry will be prepared from the blood samples and probed as follows: Tube 1 → CD11c-APC, MHCII-FITC, Gr-1-PE and CD11b-PerCP. Tube 2 → CD3-APC, NK1.1-FITC, CD115-PE and B220-PerCP/Cy5.5.

After flow cytometric analysis, gating of the different cell types will be done as follows: T cells → $CD3^+$ $B220^-$, B cells → $CD3^-$ $B220^+$, NK cells → $CD3^-$ $NK1.1^+$, Monocytes → $CD115^+$, Neutrophils → $CD11b^+$ $Gr\text{-}1^+$ and Dendritic cells→ $CD11c^+$ $MHCII^+$.

Next, we will use a protocol adapted from Butcher et al. to analyse immune cells in the aorta/plaque itself. After the creation of a single cell suspension from the aorta/plaque using a combination of collagenase I and XI, hyaluronidase type I and DNase, cells will be passed through a cell strainer and will be resuspended in buffer for flow cytometry and Fc blocked.

Single cells will then be stained for the common leukocyte marker CD45 and further stained with the ImmunoTools anti-mouse antibodies as described above. Using this method we can assess the infiltration of different types of immune cells in the plaque and the effect of everolimus on this infiltration. This is particularly interesting because it has been reported that everolimus negatively influences leukocyte migration into the plaque and this too has been proposed as the mechanism behind the anti-atherosclerotic effect of this drug.

Taken together, this project will help us to discover the true mechanism behind the positive impact of everolimus on atherosclerosis and the ImmunoTools anti-mouse antibodies for flow cytometry will assist us in answering at least two questions that have puzzled atherosclerosis researchers for years.

These experiments, and others, will form a solid base for mTOR inhibitors as new add-on drugs for high-risk atherosclerosis patients.

ImmunoTools *special* AWARD for **Ammar Kurdi** includes 25 reagents

FITC - conjugated anti-mouse CD3e, CD4, CD8a, CD11b, CD19, CD44, CD45, Gr-1, NK-cells, a/b TCR, g/d TCR,

PE - conjugated anti-mouse CD4, CD8a, CD11b, CD19, Gr-1, NK-cells,

APC -conjugated anti- mouse CD3e, CD4, CD11b, CD19, CD45, CD49d, Gr-1, NK-cells

DETAILS