Localization of ABC cells in spleen of old and autoimmune-prone mice

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Introduction

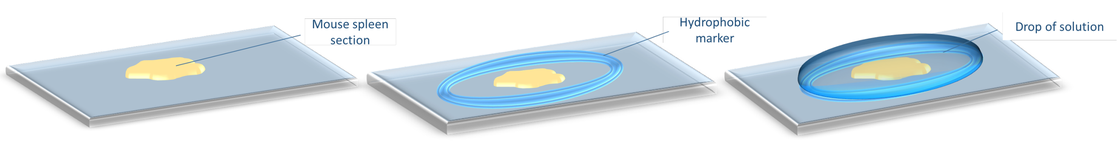
In 2011, Kira and Anatoly Rubtsov published a paper in which they described a certain population of B-lymphocytes, known as ABC. The quantity of these cells increases in old mice spleens and during the development of autoimmune diseases. In addition, it has been demonstrated that ABCs directly trigger the development of autoimmunity.

It is well known that spleen contains lymphoid follicles, divided in T-cell zone and B-cell zone, as well as the germinal centre, place of interaction of both types of cells during the immune response. So the main objective of our project is to localize and identify ABC cells in spleens of different types of mice and depending on their location define their possible functions.

Methods

For our experiments, we have used the slides with thin slices of spleen emerged in gel (8 microns).

We circled a zone around the spleen using a hydrophobic marker to prevent the drops of liquid from leaking out, keeping them on a particular part of the slide.



Then, using PBS buffer, we incubated the cells for 15 minutes. After the incubation, we added block for 1.5 hours to eliminate the non-specific bindings within the cells. Following the block, we washed the cells 3 times using the PBS buffer. For the duration of next 20 minutes, we have added the avidin and biotin proteins to prevent the non-specific bindings of biotinylated antibodies, washing the cells 3 times for 5 minutes afterwards. We have stained the cells overnight at 4C, adding the mixture with fluorescent antibodies to the slices of spleen in order to distinguish the cells of interest.

The second day of an experiment has begun with washing the cells 5 times for 5 minutes. Then we conducted the second staining for 1.5 hours to bind the fluorescent antibodies to proteins of the cells we research. We repeated the washing of the cells 5 times for 5 minutes. Subsequently, we added the mounting media to prevent the destruction of fluorescent antibodies, put the coverslip on the spleen and fixed with nail polish. The slides were analyzed with the fluorescent microscope using 10x and 40x magnifications.

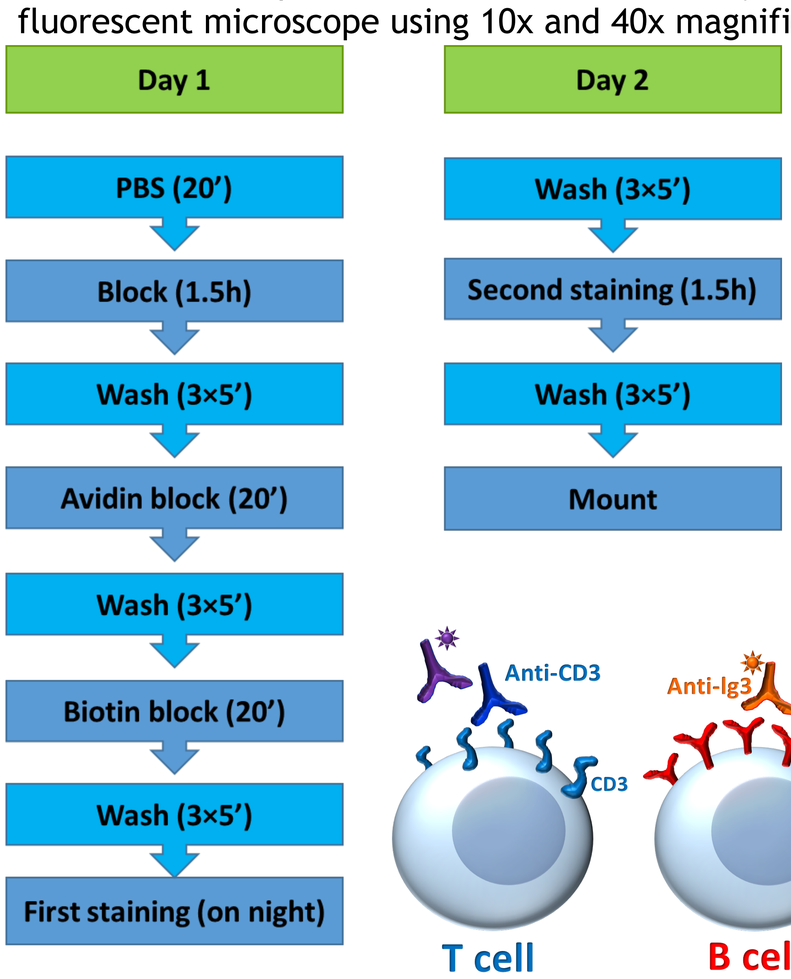


Figure 1. Methods.

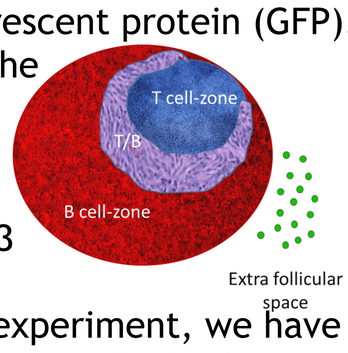
Figure 2. Schematic of staining with fluorescently labeled antibodies against B and T cells.

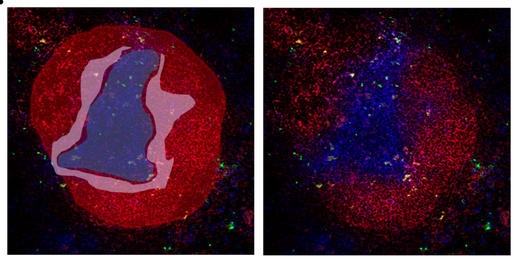
Experiment

healthy and mice that are subject to the lupus-like autoimmune diseases. The immune cells of our interest are located in the spleen follicles; thus, we were specifically interested in the research of this organ. Particularly, we researched the localization of the ABC cells relative to the B- and T-cell zones in the lymphoid follicles. All mice were genetically modified in a way that the Age-Associated B-cells contained the green fluorescent protein (GFP).

GFP has allowed us to detect the cells using a microscope.

The cells in B- and T-zones were dyed red (using IgD antibodies) and blue (using CD3 antibodies) correspondingly.





During the second part of the experiment, we have determined the location of ABC relative to the germinal center of a follicle – the place of B- and T-cells interaction during the phase of active immune response. To determine the localization of the germinal centers, the samples were dyed with the PNA reagent.

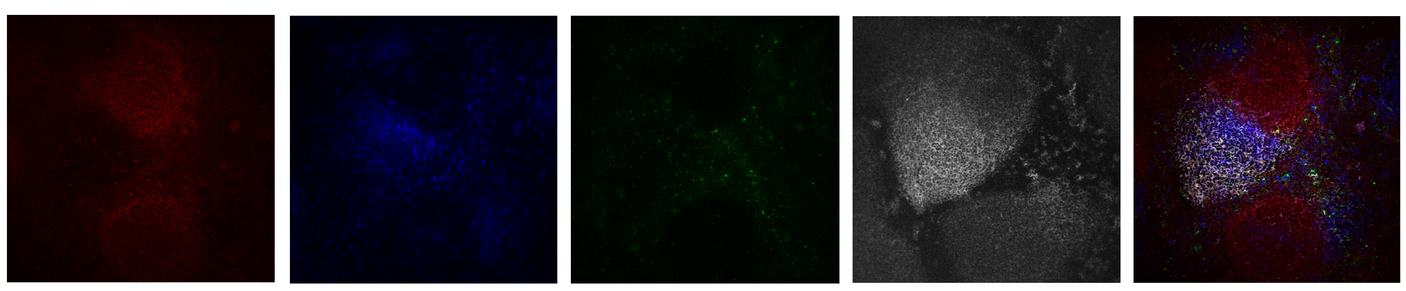


Figure 3. Staining of spleen section with individual fluorescence antibodies.

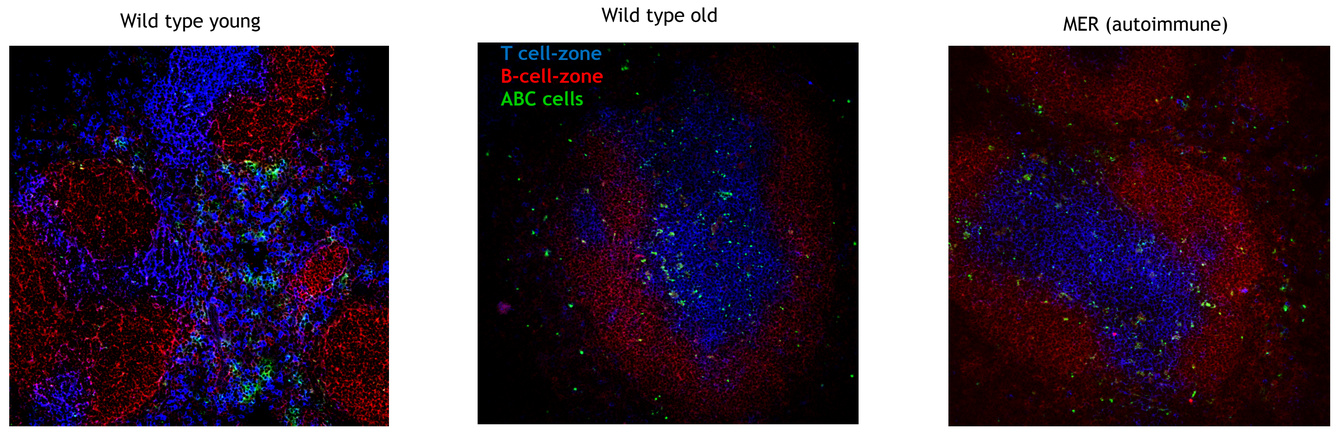
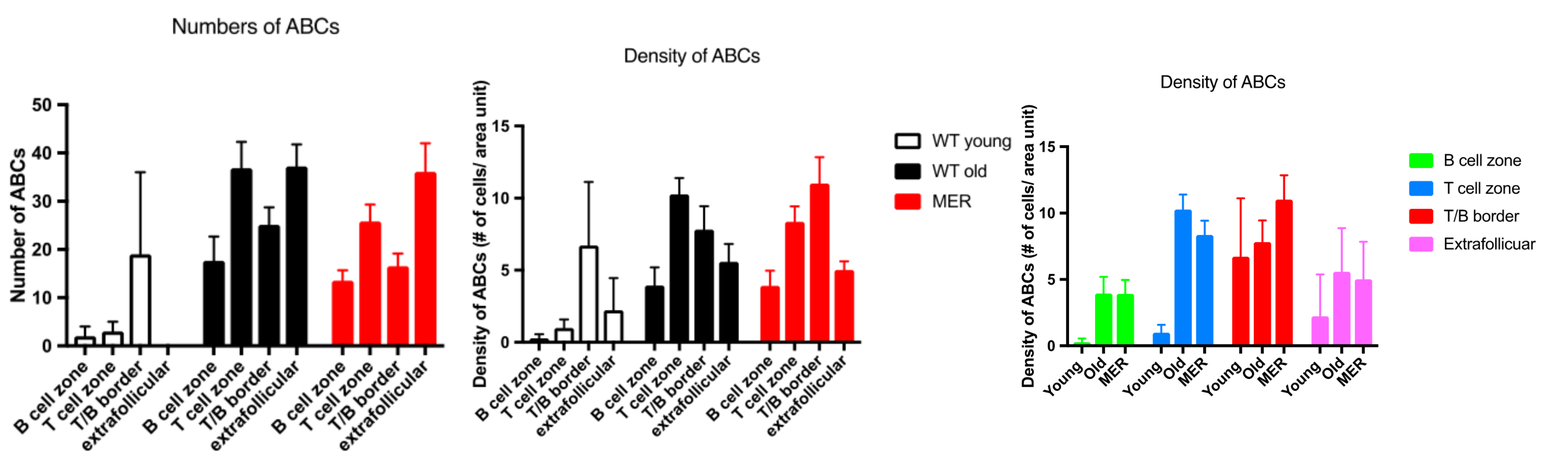


Figure 4. Fluorescent microscopy staining of spleen sections obtained from wild type young, wild type old and MER (autoimmune) mice.



Results and conclusions

- In young B6 mice the majority of ABCs are in T/B border.
- In old and MER mice the majority of ABCs are found in T and T/B zones.
- In MER mice the majority of ABCs are located in T/B zone while in old mice they are in T zone
- In old and MER mice the quantity of ABCs in extra-follicular zone is significantly bigger compared to young B6 mice.
- ABCs are not found in Germinal Centers, but more samples should be analyzed in order to confirm it.

After analyzing the different results we made several conclusions about the localization and possible function of ABCs:

- Upon the progression of age or development of disease, ABCs increase in number and change their localization from T/B border to T cell zone, which suggest that they might interact with T-cells to amplify immune response.
- ABCs increase in extra-follicular space suggest that they become antibody producing cells and directly affect the development of autoimmunity.