Imbalance or decreased efficacy of adaptive immune responses partially serves as a basis of still growing number of patients suffering from cancer or autoimmune diseases. Thus far, leukocytes were in the center of interest for the majority of immunologists. This allowed us to understand molecular mechanisms governing their development, function under physiological and pathophysiological states, finally led to development of novel therapies e.g. checkpoint inhibitors – drugs that opened new avenue to fight cancer in otherwise untreatable patients. Currently, it becomes evident that function of leukocytes highly relies also on other lymphoid cells – fibroblastic reticular cells (FRCs) in particular. Hence, view on FRCs, immunologically specialized myofibroblasts that comprise as many as 20-50% of cells of non-hematopoietic cell compartment in lymph nodes (LNs), has rapidly switched from bystanders to key immunomodulators of the adaptive immunity. Immunomodulatory role of FRCs was already reported at every step of adaptive immunity, from its development, through the maintenance and finally suppression of effector T cell proliferation within LN itself to prevent damage to its infrastructure. FRCs create exceptionally complex network for migration of both leukocytes and soluble antigens and signaling molecules. Interestingly, FRCs, through the expression of podoplanin (PDPN), constantly contracts which compress LN and reduce their size under physiological conditions. Upon immunization, PDPN-mediated contraction is inhibited, leading to expeditious LN relaxation (which corresponds with LN swelling observed during illness) that precedes and generates space for rapid lymphocyte proliferation. Strikingly, impairment of PDPN in mice leads to significant increase in markers of development of adaptive immune response.

Equally interesting studies show that the efficacy of our adaptive immune response might rely on time of the day, when the immunization/infection has occurred. As it usually takes weeks to fully develop an adaptive response, this effect is rather surprising. As studied by Druzd et al. on mouse models, markers of activation of adaptive immunity are significantly increased in many models used (experimental autoimmune encephalomyelitis, infection with H. pylori and influenza A virus) if the immunization/infection occurs in the late light (rest) phase vs. late night (active) phase. This effect seems to be true also for humans, as a recent influenza vaccination trial in elderly patients (ID:ISRCTN70898162) has shown that vaccination early in the morning (late rest phase) could increase the antibody serum concentration when compared to the vaccination in the afternoon.

Taken together the emerging role of FRCs and circadian rhythms in the development of adaptive immunity responses, we asked the question if the network of FRCs changes significantly throughout the day and shows circadian rhythmicity and whether states of maximal and minimal contraction/relaxation will affect efficacy of development of adaptive, humoral response. Noteworthy, our preliminary results presented that there is strikingly significant difference between size and mass of LNs dissected from wildtype mice late at night (end of their active phase) when compared to LNs dissected at day and early night. Thus, we will study how does the ~70% increase of LNs mass within as little as 6 hours (early vs. late night observation) impacts network of FRCs. We will develop state of the art imaging technique that makes the LN tissue both transparent and expanded. The obtained transparency will allows us, for the first time, to visualize the entire network of FRCs (as the microscope laser will be able to pass the entire specimen), while the expansion will guarantee separation of densely packed FRCs during image analysis. Finally, we will find states of maximal and minimal relaxation of FRCs during the day and study how does it affect efficacy of mice immunization/vaccination.

Presumably, our findings will (1) equip immunologists with a powerful tool to study cell-to-cell interactions within the entire lymph nodes, (2) reveal circadian rhythmicity of changes in FRCs network structure and (3) prove that state of FRCs contraction/relaxation during the immunization affects its efficacy. We believe such inspections might enhance our understanding of early events during generation of adaptive immune response and, potentially, lead to development of new strategies for the enhancement of adaptive immune response.