Changes of podosomes morphology in the macrophages (RAW 264.7) as an indicator of inflammation (M1) or repair (M2) phenotype

Iwona Lasocka[a], Lidia Szulc-Dąbrowska[b], Zuzanna Biernacka[b], Ewa Skibniewska[a], Michał Skibniewski[c], Marie Hubalek-Kalbacova[de]

[a]Department of Biology of Animal Environment, Faculty of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland, [b]Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland, [c]Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland, [d]Institute of Pathological Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic, [e]Faculty of Health Studies, Technical University of Liberec, Liberec, Czech Republic

Introduction: Podosomes (**PDs**) are cell adhesions structures that play a pivotal role in cell migration, mechanosensing, cell-matrix attachment, extracellular matrix degradation. Their morphology changes depending on the alterations in the surrounding microenvironment (e.g. substrate on which cells migrate or cell contact with foreign antibodies). Based on these changes, it is possible to assess the strength of adhesion, the speed and type of migration, and the reaction to foreign factors. The ability to identify specific changes in PDs morphology may become a valuable method for assessing the interactions of cells, primarily from the immune system, with various materials, crucial, for example, in regenerative medicine. Because PDs architecture may determine many immune function of macrophages, **the aim of the present study** was to identify PDs morphology in M0, M1 and M2 RAW 264.7 macrophages.

Material and methods: M1 and M2 macrophages were generated by classical (IFN-γ and LPS) or alternative activation (IL-4 and IL-13), respectively, whereas M0 macrophages were cultured in a complete medium without addition of any factors (Figure 1). M1 and M2 macrophages were distinguished by the expression of NOS2 and arginase-1, respectively, using intracellular staining and flow cytometry analysis (Figure 2). PDs architecture in M0, M1 and M2 cells was assessed based on the distribution of actin and vinculin using immunofluorescence staining and fluorescence microscopy analysis.

Results: All phenotypes of RAW 264.7 macrophages presented the presence of PDs organized in clusters, rosettes and rings, but without podosomes belts (Figure 3). In M1 also single podosomes were identified and located in different parts on the ventral cell surface. Moreover, stimulation with LPS and INFγ resulted in further cell spreading and appearance of structures resembled podosomes fusion with a strongly marked ring of actin and peripheral accumulation of vinculin.

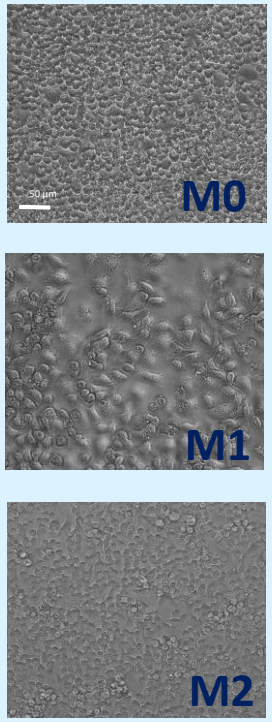

Fig. 1. Representative photos of BMMs after 48-hour culture on glass coverslip.

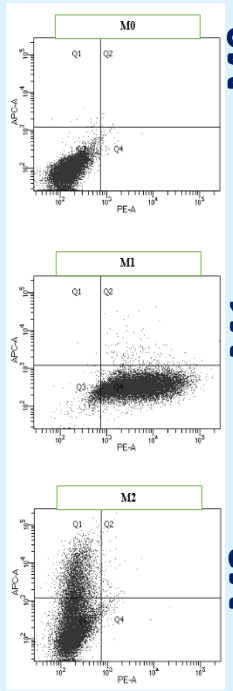

Fig. 2. Representative cytograms of the expression of NOS2 (PE) and arginase-1 (APC)

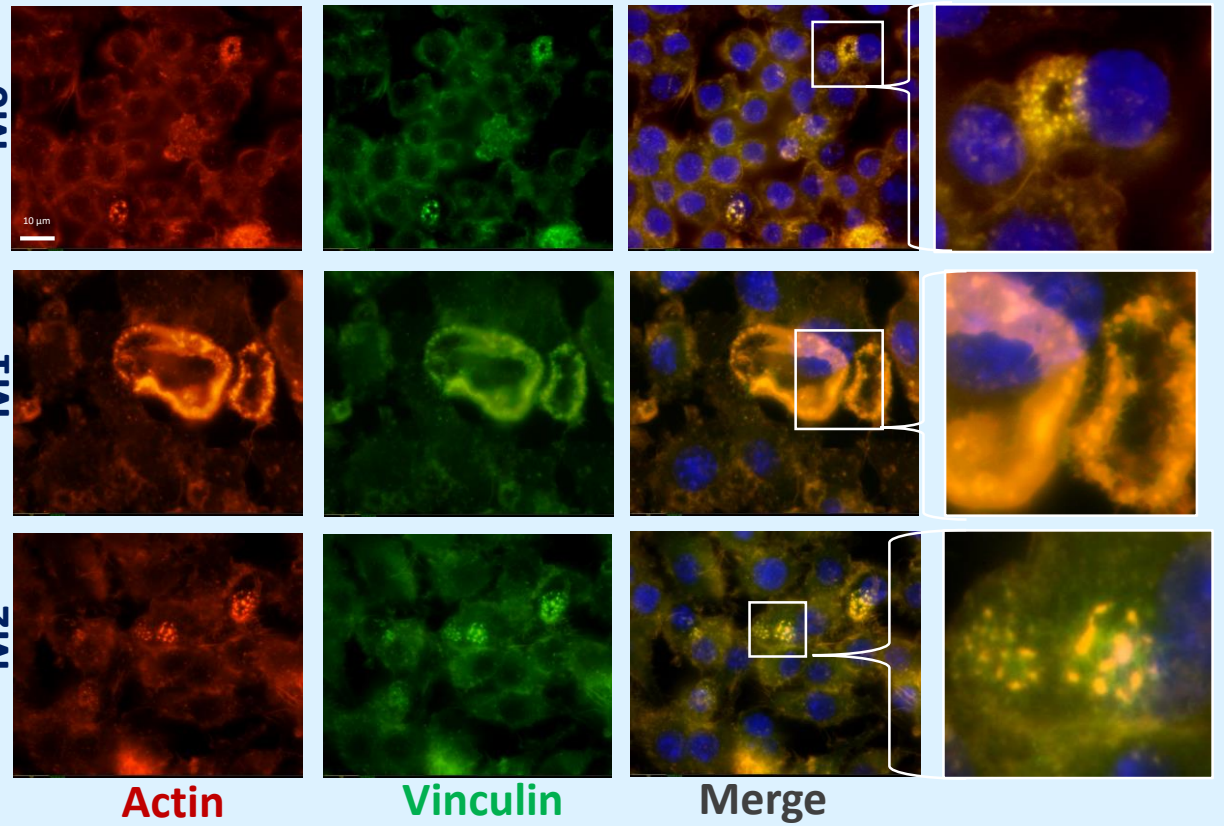

Fig. 3. Representative photos of structures formed by podosomes and FAs in BMMs (M0 and M2) after 48-hour culture on glass coverslip. Red – actin, Green – vinculin, Blue - DNA.

Conclusion: Our hypothesis assumes that macrophages treated with LPS for 24 h and INFγ for 48 h responded to inflammatory stimulation and organized the ring-shaped podosomes-like structures in RAW 264.7 cells. Taken together, our results indicate that the differences between M1 and M2 also apply to formation and organization of PDs in RAW 264.7 macrophages.

This work was supported by the National Science Centre in Cracow, Poland (grant no. 2022/06/X/NZ3/00289).