PhD Thesis Defense ALBERTO SOSA COSTA 'Insights on the Spation-Temporal Organization of Integrins and their Ligands Using Quantitative Biophysicac Tools'

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Thursday March 2, 11:00. ICFO Auditorium

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Single Molecule Biophotonics

ICFO-The Institute of Photonic Sciences

The migration of leukocytes from the blood stream to sites of injury and infection in the extravascular tissues is fundamental for the immune response. Two of the main receptors

mediating this process are the integrins ?L?2 and ?4?1, both expressed on the leukocyte cell membrane, which bind to their respective ligands ICAM-1 and VCAM-1, expressed on endothelial cell (EC) membrane. The dynamic and lateral organization of integrins on the cell membrane has been shown to be crucial in the regulation of cell adhesion. Likewise, organization of ligands in small domains (clusters) would probably reinforce the bonds formed with the integrins, thus increasing leukocyte adhesion. However, how the spatiotemporal behavior of integrins and their ligands is affected by the influence of external factors, such as mechanical and/or biochemical stimuli, has not been extensively studied. In addition, the impact of such spatiotemporal changes in the process of leukocyte adhesion and migration has not been addressed yet. The main aim of this thesis has been to address these questions using a combination of state-of-art biophysical tools, including advanced cell imaging, single molecule dynamic approaches, cell mechanical stimulation, and custom-designed algorithms for data quantification.

From the technical side, our general approach involved the use of single particle tracking (SPT) approaches to monitor the dynamics of individual molecules implicated in the process of the cell adhesion, in combination with different super-resolution microscopy techniques, such as STED and STORM, to visualize changes in their nanoscale organization upon the influence of biochemical and/or mechanical stimuli. To mechanically stimulate cells we developed two different approaches; namely, a mechanical stretching device and a parallel-plate flow chamber (PPFC). We integrated these devices into our single molecule set-up and succeeded in recording the diffusion of integrins on cells plated on the mechanical stretching device upon varying stress conditions. As part of this thesis, we also applied different custom-made data analysis algorithms existent in the Lab and developed novel algorithms aimed at tracking and quantifying changes in the migratory behavior of T-cells on ECs exposed to shear stress.

Using this powerful palette of tools we discovered that, as a consequence of prolonged shear flow exposure, ICAM-1 undergoes a global reorganization on the EC membrane accompanied by the formation of ICAM-1 nanoclusters. These nanoclusters were found to co-localize with shear flow-induced actin-enriched patch-like structures. Moreover, we showed that T-cells migrate faster and interact for shorter period of times on ECs mechanically stimulated as compared to ECs not subjected to shear stimulation. Hence, from these results, we concluded that continuous shear flow regulates the spatial organization of cell adhesion receptors on ECs, which in turn modulates leukocyte migration. In addition, we showed that chemokine (CXCL12) stimulation leads to rapid and transient activation of the ?4?1 expressed on T cells. These changes in activation profile directly correlate with talin recruitment,

restricted lateral diffusion and integrin immobilization. Moreover, co-stimulation with CXCL12 and the ligand VCAM-1 potentiated integrin immobilization. In addition, superresolution imaging revealed that the nanoscale organization of ?4?1 remains unaffected upon CXCL12 and/or VCAM-1 stimulation. Our data, thus, indicate that docking by talin of the chemokine-activated ?4?1 to the actin cytoskeleton favors integrin immobilization, which likely facilitates ligand interaction and increased adhesiveness. The overall finding of this thesis indicates that cells of the immune system respond to mechanical and biochemical stimuli by rapidly readjusting the spatiotemporal behavior of integrins and ligands on the cell membrane modulating in turn cell adhesion and migration.

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