EXTRACTION OF THE PROTEOME OF THE IMMUNOLOGICAL SYNAPSE IN NK CELLS

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Background: NK cells are innate immune cells that provide protection against virally compromised or transformed cells. Direct killing occurs through the release of lytic granules onto the target cell at the Immunological Synapse (IS). The IS is a unique structure formed at the site of contact between the immune cell and the target cell and is characterized by a dense mesh of actin on the effector cell side. While many key components regulating the dynamics of the IS have been identified, its complete composition remains unknown and our understanding of this structure remains therefore limited to the list of knowns targets with available antibodies. To address this, we sought to create an innovative experimental approach to identify new molecular components mobilized on the NK cell side of the IS during cytotoxic activity.

Materials/Methods: We have developed a method to extract and purify the portion of the NK cell cortex engaged in the IS. Ligand-coated beads replaced typical target cells and were incubated with a human NK cell line (NK-92), allowing the IS to form. Using a combination of cross-linkers, detergent and sonication, we then separated and purified the cellular cortex at the IS from the bulk of the NK cell.

Results: Firstly, to demonstrate that our technique works, we screened our lysates for a series of known markers for the IS and other various cellular compartments to testify to the specificity and efficacy of our purification approach. In parallel, we observed by immunofluorescence that actin was maintained as a patch on the beads following sonication of the whole cell. The footprint left by the effector cell onto the bead suggests that the architecture of the IS was preserved by our extraction protocol, increasing the probability of purifying intact cortices. In a subsequent large-scale assay, we have analyzed by mass spectrometry the proteome ("immune synapsome") present at the IS and compared it between activated and tethered-only NK cell conditions. 515 proteins were unique to the cortex of activated NK cells and 256 additional ones were enriched compared to those in the tethered-only cortex condition. Among the proteins enriched, we confirmed the identification of expected IS-localized proteins (CD3ζ, ZAP70, granzymes, perforin).

Conclusions: This initial result validates our method and provides confidence to further investigate other IS-enriched proteins, with the ultimate goal of uncovering novel key regulators of NK cell cytotoxic function.