MOI Project 17

The role of the purinergic receptor P2X7 for cross-presentation of antigens during poxviral infection

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Vaccinia virus (VACV) belongs to the poxvirus family and is a large dsDNA virus that replicates in the cytoplasm of the infected cell. It encodes more than 200 genes, about a quarter of which have immunomodulatory functions. The virus is an acute infectious agent and has been successfully used in the past as a vaccine for the eradication of smallpox (variola). Attenuated VACV strains such as MVA (modified vaccinia virus Ankara) are tested in clinical trials as recombinant vector vaccines against infectious diseases for their safety and immunogenicity. We have shown that cross-presentation plays an essential role for optimal cytotoxic CD8+ T cell (CTL) responses in this infection model. Cross-presentation enables cell-associated antigens from the extracellular milieu, which are normally presented on MHC class II molecules, to also be presented on MHC class I molecules. This process enables a combined and therefore more efficient CD4+ and CD8+ T cell response to various infectious agents. The investigation of the relevance of cross-presentation for the induction of T cell responses in the VACV infection model in the mouse is a central topic of the research group. In previous work we were able to show that the ability to cross-present is not only determined by the type of cell that presents, but also by the infected cell that provides the antigens. These so-called feeder cells licence bystander non-infected cells to take up the antigens and cross-present them. This process requires both, effector molecules of the innate immune system such as type I interferons as well as components of the signalling pathway for apoptosis such as the purinergic receptor P2X ligand-gated ion channel 7 (P2RX7).

As a surface receptor, P2RX7 is mainly expressed in endothelial, epithelial and immune cells. It is involved in the regulation of immune responses via cytokine release, inflammatory processes through inflammasome activation and apoptosis through regulation of mitochondrial activity. Studies show that P2RX7 is also localised intracellularly on mitochondrial structures. The absence of functional P2RX7 in infected feeder cells therefore resulted in a greatly reduced number of cross-presenting dendritic cells (DC), which contained significantly fewer virus-specific peptide/MHC-I complexes on the cell surface. The latter also explains the significantly reduced cytokine production (IFNγ, TNFα) of virus-specific CD8+ T cells co-cultured with these cross-presenting DC. In this project, the cellular signalling pathways and molecular processes relevant for efficient cross-presentation will be investigated as a function of P2RX7, both in the feeder cell and in the cross-presenting DC.

The planned working program includes the investigation of metabolic processes in the feeder cell with relevance for cross-presentation depending on P2RX7, such as energy metabolism or mitochondrial activity. Colocalisation studies on the intracellular localisation of P2RX7 during infection using confocal microscopy (CLSM). Analysis of the secretome (proteins, nucleic acids) of infected feeder cells in relation to P2RX7 in the supernatant and in extracellular vesicles. Functional analysis of the vesicular fractions e.g. for cross-presentation. Further analysis of the vesicles with measurement of particle size, concentration and content (see above). Investigation of cell biological changes in the formation of EVs after infection (CLSM). Investigation of the role of the inflammasome as a function of P2RX7. Co-expression of P2RX7 in recombinant MVA for in vitro and in vivo analyses after vaccination in the mouse model.