Project 17: Characterization of vaccinia virus-encoded autophagy inhibitors

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Background:

Autophagy is a process that is activated under various conditions of cellular stress or infection to ensure the survival of the cell or drive it into apoptosis. Intracellular pathogens such as viruses have developed mechanisms to use autophagy for their replication or to antagonize autophagy-mediated antiviral effects such as digestion of viral particles. In addition, autophagy plays an important role as a component of the adaptive immune system in antigen processing and presentation for T cells. Vaccinia Virus (VACV) is a large dsDNA virus. It leads to an acute viral infection and has been successfully used as a vaccine for the eradication of smallpox. Vaccines based on attenuated strains such as MVA (modified Vaccinia Virus Ankara) are tested in clinical trials due to their safety and immunogenicity as recombinant vector vaccines in infectious diseases and in the immunotherapy of tumors.

Own previous work:

We could show that autophagy in MVA infected cells contributes significantly to MHC class II antigen processing and presentation and thus to CD4+ T cell activation (1). In addition to canonical autophagy, there are alternative, non-canonical activation pathways, which are much less well understood in molecular and functional terms (2). Only recently, the induction of autophagy by exogenous cyclic GMP-AMP (cGAMP) has been described in a non-infectious context (3). The cGAS-cGAMP-STING activated autophagy is based on a new mechanism independent of ULK1/2 that requires WIPI2 and ATG5 among others and shows great similarity to STING-dependent autophagy, which is induced in MVA infection but actively inhibited by virulent strains such as WR. By siRNA screening we have already been able to identify inhibitory viral gene products of strain WR that prevent or significantly delay the formation of autophagosomes.

Aim of the project:

In this project, the viral antagonists of STING-dependent non-canonical autophagy will be molecularly and functionally characterized. The cellular interaction partners will also be identified and the novel activation pathway will be further investigated using immunological, molecular and cell biological methods. A detailed knowledge of the function of the viral antagonists will also contribute to the identification of new target structures for immunotherapeutic approaches and to the improvement of viral vector vaccines.

Work program:

CRISPR/Cas9 mutagenesis for the generation of WR deletion mutants (•WR) and MVA-BAC mutagenesis for the generation of the revertant MVA (revMVA). Detailed *in vitro* and *in vivo* characterization of mutants and revertants with regard to immunogenicity and the ability to induce or inhibit autophagy. Furthermore, identification of cellular interaction partners for STING-dependent induction of non-canonical autophagy after infection. Methods: westernblot analysis, immuno-precipitation, FACS (immune) analysis, CLSM, siRNA, CRISPR/Cas9 mutagenesis, intracellular cytokine staining, T cell activation, vaccination, KO mice, yeast 2-hybrid screen, proximity ligation assays, structural analysis.

1. Thiele, F., Tao, S., Zhang, Y., Muschaweckh, A., Zollmann, T., Protzer, U., Abele, R., & Drexler, I. (2015) *J Virol* **89**, 2698-709
2. Tao, S. & Drexler, I. (2020) *Front Immunol*, in press
3. Gui, X., Yang, H., Li, T., Tan, X., Shi, P., Li, M., Du, F. & Chen, Z.J. (2019) *Nature* **567**, 262-6.