Improvement of MVA-based Vaccines by Expression of an Autophagy Inhibitor

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Abstract

Autophagy is a lysosomal degradation pathway playing a crucial role in immunity. In the context of vaccination strategy based on Modified Vaccinia virus Ankara (MVA), autophagy is likely to promote antigen specific immunity by its involvement in antigen processing and presentation. However, it could also limit vaccine efficacy due to its antiviral activity. The study presented here assesses the interplay between autophagy and MVA-based vaccine. After confirming MVA-based vaccine increased autophagy, a transgene coding for an autophagy inhibitor was inserted into MVA-based vaccines. The autophagy inhibitor chosen inhibits late stage autophagy by blocking fusion between autophagosomes and lysosomes. Expression of autophagy inhibitor did not affect MVA-based vaccine production or expression. Specific cellular immune responses detected by interferon γ ELISpot against the vector or exogenous antigens were similar following vaccination with a MVA-based vaccine expressing or not autophagy inhibitor. However, surprisingly, using MVA-based vaccine expressing autophagy inhibitor for therapeutic vaccination in a mouse tumor model led to an improvement of mice survival compared to basic MVA-based vaccine. Such improvement was confirmed in a different tumor model. Injection of MVA vector expressing only autophagy inhibitor without antigen had no effect on mice survival, indicating that autophagy inhibitor had no antitumor activity per se and that antigen expression was necessary. These results demonstrate that targeting autophagy pathway is a new approach to improved vaccine efficacy; however further studies are needed to fully understand the mechanism by which blocking autophagy flux leads to a stronger vaccination effect.

Experimental Strategy

Expression of a Protein to Block Autophagy Induced by MVA-based Vaccine

Autophagy inhibitor : M2 S60 (N-terminal 60 amino acids of influenza A virus M2 protein). M2 S60 blocks the fusion between lysosome and autophagosome (Gannage, Dormann et al., 2009).

MVA-based vaccine: Two model antigen : β-galactosidase and E7 from HPV16

MVA increases autophagy flux.

Assess the impact of autophagy on MVA-based vaccine efficacy.

Vaccine Characterization

Vaccine Expression

Expression kinetics of MVA-based vaccine. MRCS cells were infected with indicated MVA-based vaccine (MOI=0.1). Infections were stopped at different times post-infection and cell lysates were analyzed by Wb using anti-β-gal (Novus) and anti-Flag (Sigma) antibodies.

Autophagy Inhibition

M2 S60 blocks LC3-II turnover. MRCS were infected with indicated MVA (MOI=3) during 24 hrs +/- chloroquine during the last 30 min of infection. Cell lysates were then analyzed by Wb using anti-LC3 antibody (Novus). Incubation time 5 hours post infection.

Vaccine replication

MVA replication is not affected by M2 S60 expression. DC51 cells were infected with indicated MVA (MOI=0.1). At different times post-infection, cells and supernatants were harvested and amount of viral progeny was determined by plaque assay on DF1.

Autophagy inhibitor, M2 S60, is expressed, functional and does not impact virus production.

Cellular specific immunity is similar with or without M2 S60. M2 S60 expression improves specifically vaccine efficacy.

References


Conclusion

Degradation inhibition of autophagosomes generated by MVA-based vaccine increases vaccine efficacy.