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

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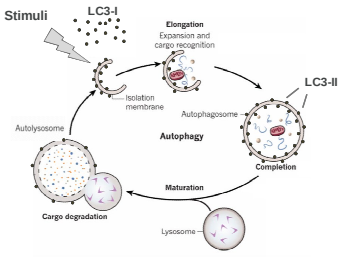
Abstract

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 antigen 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 demonstrated 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.

Autophagy and Immunity

Autophagy cycle



Adapted from Levine, Mizushima et al., 2011

Autophagy Role In Innate Immunity

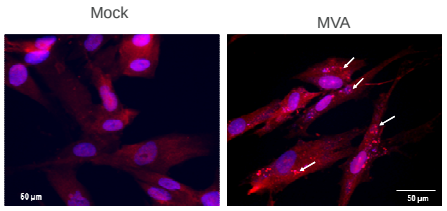
- Direct Elimination of Intracellular pathogen
- PRR (TLR) Effector Mechanism
- Autophagic Macrophage Activation
- Enhanced Type I IFN production Through Delivery of Viral nucleic Acids to Endosomal TLRs
- Cooperation with immunity-related GTPases

Autophagy Role in Adaptative Immunity :

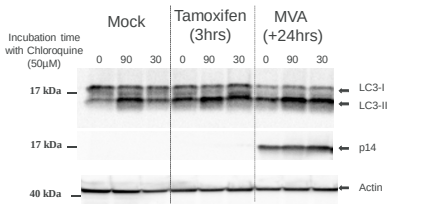
- Th1 effector Mechanism
- Antigen presentation (MHC I and MHC II)

Adapted from Deretic & Levine, 2009

Autophagy and MVA



MVA infection increases autophagic vacuoles. Twenty-four hours after MVA infection (MOI=3), MRC5 cells were stained with DAPI (blue) and LC3 antibody (Sigma) followed by Cy-3 conjugated secondary antibody (red). Arrows indicate LC3-positive punctua characteristic of autophagic vacuoles



MVA infection increases autophagy flux. MRC5 were infected with MVA (MOI=3) for 24 hrs, chloroquine (an inhibitor of autolysosomal degradation) was added 90 or 30 min before stopping infection. LC3-I/LC3-II profile was assessed by Western blot analysis (W.B). MRC5 treated with tamoxifen (autophagy inducer) were used as a positive control. Anti-Actin (Sigma) and anti-p14 (non commercial, rabbit polyclonal) antibodies were used as control of protein loading and virus infection respectively.

MVA increases autophagy flux.

Objective

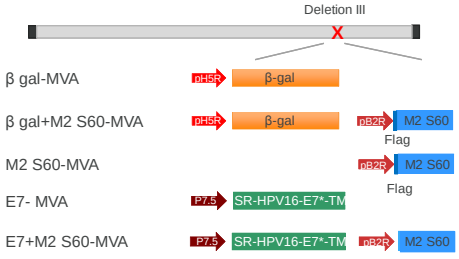
Assess the impact of autophagy on MVA-based vaccine efficacy.

Experimental Strategy

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

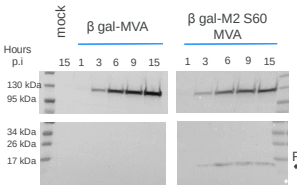
Autophagy inhibitor : **M2 S60:** N-terminal 60 amino acids of Influenza A virus M2 protein. M2 S60 blocks the fusion between lysosome and autophagosome (Gannage, Dormann et al., 2009).

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



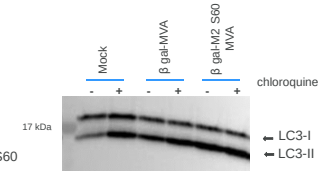
Vaccine Characterization

Vaccine Expression



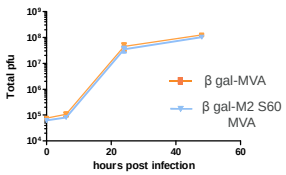
Expression kinetics of MVA-based vaccine. MRC5 cells were infected with indicated MVA-based vaccine (MOI=3). Infections were stopped at different times post-infection and cell lysates were analyzed by W.B using anti-β gal (Novus) and anti-Flag (Sigma) antibodies.

Autophagy Inhibition



M2 S60 blocks LC3-II turnover. MRC5 were infected with indicated MVA (MOI=3) during 24 hrs +/- chloroquine during the last 90 min of infection. Cell lysates were then analyzed by W.B using anti-LC3 antibody (Novus)

Vaccine replication

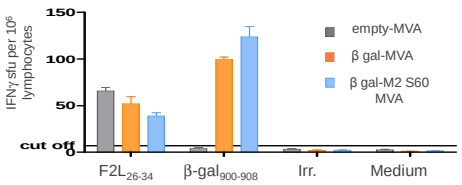


MVA replication is not affected by M2 S60 expression. DF1 cells were infected with indicated MVA (MOI=0,1). At different times post-infection, cells and supernatants were harvested and amount of viral progeny was determined by plaque assay on DF1.

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

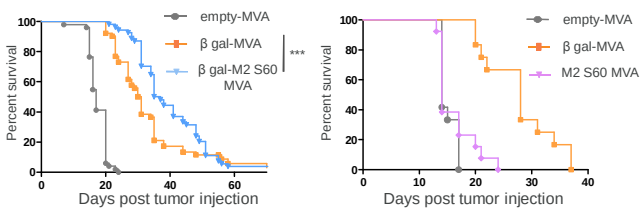
Vaccine efficacy

Immunogenicity of MVA-based Vaccine (β-gal)



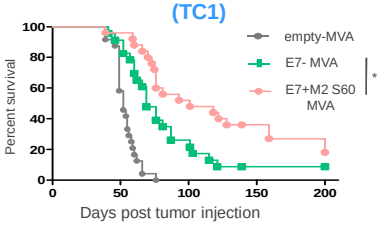
M2 S60 expression does not modify cellular immune response against MVA and β-gal antigens. BALB/c were immunized i.v on d0 and d+7 with 10^3 pfu of indicated MVA. On d+14, lymphocytes isolated from splenocytes were stimulated overnight with indicated peptides and IFN γ secreting forming units (sfu) were measured by ELISpot. Histograms represent mean and SEM of two independent experiments done in quadruplicate with 5 mice per group.

Vaccine Efficacy on β-gal-expressing Colon Carcinoma Model (CT26CL25)



M2 S60 expression improves efficacy of therapeutic MVA-based vaccine using β-gal as model antigen. CT26CL25 ($2x10^5$ cells) were injected by i.v.; 2 and 9 days later MVA-based vaccine (10^3 pfu) were injected i.v. The Kaplan-Meyer survival curves illustrate the result of four pooled experiments with a total of 52 mice per group for the comparison of β-gal-MVA to β-gal+M2S60-MVA, and one experiment with 13 mice per group for the comparison of β-gal-MVA to M2S60-MVA. Mice survival was analyzed using Wilcoxon test (*** P < 0.005).

Vaccine Efficacy on HPV16-E6-E7-Expressing Lung Cancer Model (TC1)



M2 S60 expression improves efficacy of therapeutic HPV16 E7 MVA-based vaccine. C57BL/6 mice were injected i.v by $2x10^5$ TC1 cells; 7 and 14 days later MVA-based vaccine (10^3 pfu) were injected i.v. The Kaplan-Meyer survival curves illustrate the result of two pooled experiments with a total of 24 mice per group. Mice survival was analyzed using Wilcoxon test (* P < 0.05).

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

References

Deretic V, Levine B (2009) Autophagy, immunity, and microbial adaptations. Cell Host Microbe 5: 527-49
Gannage M, Dormann D, Albrecht R, Dengjel J, Torossi T, Ramer PC, Lee M, Strowig T, Arrey F, Conenello G, Pypaert M, Andersen J, Garcia-Sastre A, Munz C (2009) Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. Cell Host Microbe 6: 367-80
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Conclusion

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