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Poster presentation

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Background

Recombinant MVA expressing HIV genes are currently being tested in phase I and II vaccine trials. During efforts to make rMVA/HIV candidate vaccines, we found variable stability of inserted gene expression. Maintaining stability upon repeated passage for vaccine production while keeping high expression of inserted gene for increased immunogenicity is highly desirable. In continuation of efforts to improve stability, we now compare three insertion sites in the MVA genome using the same HIV env gene.

Methods

Clade D HIV/UGD env gene controlled by vaccinia mH5 promoter was inserted into three regions of the MVA genome, differing in importance of the flanking DNA for vaccinia virus replication. rMVA viruses expressing this HIV Env were made and analyzed for stability and growth after serial passage by immunostaining.

Results

We analyzed three insertion sites for an HIV env in MVA: del II and del III sites and between essential genes I8/G1. Large deletions of HIV env and MVA flanks resulted from repeated passages of rMVA/HIV Env in del II region with overgrowth of non-expressing virus, indicating that del II is a problematic insertion site. Analysis of genes flanking this region identified no essential genes or genes affecting viral replication, providing a large target for viable deletion mutations. HIV env inserted between I8/G1 essential genes resulted in stable rMVA virus, since deletions extending into the essential MVA flanks would not be viable. MVA del III region analysis determined genes adversely affecting virus replication within 1-2 kbp flanking the insertion site. A vector was constructed that removed intervening non-essential sequences flanking the insertion site. rMVA/HIV Env made with this vector demonstrated env gene stability in this restructured del III site.

Conclusion

For more stable rMVA construction, insert genes between essential genes or genes affecting viral replication. Avoid sites between non-essential genes that provide targets for viable deletions.