Development of viral and bacterial vehicles for antigen delivery into mammalian cells

Abstract

We have recently developed an antigen delivery vehicle based on the mouse polyomavirus-like particles, produced in baculovirus expression system, and carrying EGFP model protein inside pseudocapsids (EGFP-VLPs). Chimeric EGFP-VLPs entered mouse and human epithelial cells, fibroblasts and human and mouse dendritic cells efficiently (Bouřa at al., 2005). The role of viral structural proteins in the induction of adaptive immune responses is poorly understood. To address this issue, we focused on the effect of VP1 and chimeric "green" polyomavirus-like particles on mouse bone marrow-derived dendritic cell (BMDCs) activation. In vitro, VP1 VLPs, as well as EGFP-VLPs caused upregulation of phenotypic markers – MHCgp II and co-stimulatory molecules CD80 and CD86 in dose-dependent manner. Moreover, exposure to polyomavirus-like particles effectively induced a strong proinflammatory cytokine IL-12 secretion by BMDCs.

Here, we also show that BMDCs loaded by EGFP-VLPs are able to induce proliferation of both antigen-specific $CD4^+$ and $CD8^+$ T cells, in contrast to naive T cells. Indeed, the induction of IL-2 and INF-γ production by T cells after *ex vivo* restimulation with EGFP-VLP-loaded BMDCs was demonstrated by quantitative enzyme-linked immunosorbent assay. In parallel experiments, rEGFP-loaded BMDCs recognized EGFP-specific T cell immunity in mice immunized by EGFP-VLPs. On the other hand, the EGFP-VLPs applied by intranasal administration also caused the induction of systemic neutralizing anti-VP1 antibodies. However, production of antibodies against transported cargo (anti-EGFP) was not detected. We also showed that BMDCs pulsed with EGFP-VLPs and simultaneously treated with endosomal acidification or proteasome inhibitors were not capable to activate T cell proliferation. These results, supported also by confocal microscopy, indicate that murine BMDCs can process and cross-present EGFP-VLPs using an endosome-to-cytosol pathway. Furthemore, we observed moderate decrease of the number of splenic $CD4^+CD25^+Foxp3^+$ regulatory T cells in immunized mice. No reduction of Treg cells was observed in lymph nodes.

More recently, we have worked on construction of an alternative antigen carrier derived from cholera holotoxin. Cholera holotoxin-like protein complex carrying EGFP fused with C-terminal domain of CTA2 subunit, instead of catalytic (toxic) A1 subunit, have been prepared and analyzed fot their potential production in insect cells. Immunofluorescent microscopy and Western-blot analyses proved efficient production both CTB and EGFP-CTA2 proteins in insect cells. CTB molecule was found to form a pentamers. For the present, purification of cholera holotoxin-like molecules using D-galactose affinity chromatography did not give satisfactory yield.