



Activation, stimulation and uptake of bacterial ghosts in antigen presenting cells.

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Bacterial ghosts have been shown to be an innovative system to prepare vaccines of various bacteria with all features of the intact bacterial cell envelopes, especially all antigenic epitopes, but also to target recombinant proteins inserted in the cell envelopes of the ghost preparations to specific antigen presenting cells. To investigate the activation of the antigen presenting cell by bacterial ghosts in more detail we studied the uptake of bacterial ghosts in dendritic porcine cells and RAW macrophages and the induction of inflammatory mediators or mediators directing the immune response in THP-1 human macrophage cell line. The synthesis of inflammatory macrophage mediators such as TNFalpha in the THP1 cell line was stimulated by a hundred-fold higher dose of ghosts from *Vibrio cholerae* than the corresponding LPS using ELISA-analysis. These results confirm in vivo experiments indicating no toxic effects of ghosts in rabbits even after intravenous administration in doses stimulating significant humoral responses. We were also able to see a significant activation of IL-12 indicated by the analysis of IL-12(p70) synthesis and IL-12(p40) mRNA accumulation. This interleukine is of special importance in the activation of cellular TH1 immune responses. A rapid uptake of bacterial ghosts in macrophages within 10-30 min could be confirmed by electron microscopy. As antigen presentation is especially effective in porcine dendritic cells (DC) and even a low capacity of antigen uptake is sufficient for an induction of immune responses we investigated uptake and activation of bacterial ghosts by DC. DC are known to be phagocytic in specific immature stages. We found a significant uptake of bacterial ghosts from *Actinobacillus pleuropneumoniae* (App) and *V. cholerae* conjugated with FITC (fluoresceinisothiocyanate) within 2 h. These data suggest that bacterial ghosts effectively stimulate monocytes and macrophages for the induction of TH1 directed immune responses and dendritic cells treated with bacterial ghosts may serve as a promising vehicle for active immunization and immunotherapy in situ.