Bacteriocin DNA nanocomplexes as immunotherapeutic carriers

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Synthetic oligodeoxynucleotides (CpG-ODNs) and bacterial DNA containing unmethylated CpG dinucleotides (CpG motifs) are clinical candidates as anti-cancer agents, immune adjuvants, anti-allergens, and stand alone immunoprotective agents. Two different classes of CpG-ODNs were identified for clinical applications. B-Class ODNs, also known as K-type CpG ODN, have phosphorothioate backbones and potent B- cell activators. A-Class ODNs (or D- type ODNs) have mixed backbone and flanking G-runs at their 3`and 5`-ends. This class mainly triggers pDC to secrete IFNα, an important anti-viral cytokine. However, clinical trials revealed that native CpG-ODNs` activities are not at the therapeutic level, leading to failure of the ongoing trials. Therefore, several different approaches have been proposed in order to alleviate these undesirable side-effects (i.e. nanoliposomes, or amphipfilic biodegradable macromolecules [1,2]). Simpler complexation compounds that provide increased stability, retained activation and improved internalization of labile molecules such as CpG motif expressing ODNs, if available in bulk and can be obtained very cheaply are very suitable carriers. Cationic peptides (enterocin A and pediocin AcH/PA-1) isolated from different lactic acid bacteria (LAB) are such compounds, since they can interact with CpG-ODNs to generate stable nanocomplexes. The bacteriocins produced by LAB, are ribosomally synthesized antimicrobial peptides. The peptide is heat-stable, and cationic [3]. Here we showed that; two candidate bacteriocins, namely, i) enterocin A and ii) pediocin AcH/PA-1 complexed with different CpG ODNs led to superior activity over free ODN counterparts.

Immunostimulatory ODN-D35 and ODN-K3, and their control ODNs in which the CpG motif was methylated or inverted, were used as CpG-A and CpG-B type ODNs, respectively. In this study, Enterocin A, produced by infant isolate of *Enterococcus faecalis* OZV and Pediocin AcH/PA-1, produced by breast milk isolate of *Pediococcus pentosaceus* OZF were used as bacteriocin sources. The complexations were adjusted to 1:2, 1:4, 1:8, ODN:Bacteriocin ratio (w/w). Single-cell splenocyte suspensions were prepared ($5x10^6$/mL) and added to each well.

Three different CpG-ODN:bacteriocins complexes (1:2, 1:4, 1:8) in four different concentrations (3, 1, 0,3 and 0,1μM) were used to stimulate the cells for 24 hr. Supernatants were collected and stored for cytokine ELISA at -20°C.

Secretion of IL-6 and IL-12 into culture supernatants were determined by ELISA. Concentrations were calculated from standard curves generated by use of known amount of recombinant mouse cytokines. All ELISA assays were performed in triplicate for each groups.

Although bacteriocins were isolated from different bacterial cultures our data suggested that they are not inducing any immune response by themselves. Upon complexation with CpG-ODNs, the nanocomplexes induced significantly much higher IL-6 and IL-12 production from mouse splenocytes. Enterocin A was found to be more effective on IL-6 secretion when used together with D or K-ODNs as compared to pediocin AcH/PA-1. Interestingly, when IL-12 induction profiles were investigated, data implicated that both bacteriocins were able to pronounce the effect of D but not K–type CpG ODNs (data not shown).

When taken together present data demonstrated that bacteriocin/DNA nanocompexes significantly augmented immunostimulatory potential of different classes of CpG motifs. These results strongly suggested that bacteriocins are suitable carriers for labile nucleic acid based agonists nanocomplaxation, and could be harnessed to improve their *in vivo* efficacy for immunotherapeutic applications.

References:

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