Delivery of TLR Ligands with Polysaccharide Nanocomplexes Enhances Immune Activation

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Abstract: You should provide a summary of your work here. Not longer than. Few sentences.

INTRODUCTION

TLR therapeutics is drawing significant clinical attention. Understanding behavior of several TLR agonists will help us to design more effective vaccine adjuvants, or immuno-therapeutic agents targeting infectious diseases as well as cancer and allergy.

MATERIALS AND METHODS

Polyasaccharide (PS) extract: *G.lucidum, Balcalı strain* (extracted at 150°C) was kind gift from Prof. Dr. Erbatur. TLR ligands (TLR3 ligand: pI:C, TLR7/8 ligand:R848 and TLR9 ligand:CpG ODN) were nanocomplexed with PS (at a 1:1 weight ratio). Mouse spleen cells and RAW 264.7 were incubated with complexes (4-42 hours), at different doses (1x, 5x, 25x and 125x) and the activation was followed by ELISA, NO Assay and RT-PCR.

RESULTS AND DISCUSSION

Stimulation of splenocytes with PS–CpG ODN complex shows synergism even at low doses

In 1x and 5x dilutions, when CpG ODN (ODN 2006) complexed with PS, a robust synergistic IL-6 induction was observed (almost >4 fold increase) compared to either CpG ODN only or PS only treatments (Fig. 1). This trend is seen even at 25x dilution but the magnitude is relatively small (data not shown).

TLR expression pattern of spleen cells

In spleen cells *tlr3*, *tlr4*, *tlr6* and *tlr7* messages were strongly upregulated after PS alone stimulation for 4h (Fig.2). In stimulation of spleen cells with PS4-R848 complex we couldn't see any enhancement in any TLR gene messages, compared to R848 alone stimulation group. This is not surprising since R848 is a very potent inducer there is no PS mediated effect on gene upregulation (at 125x dilution) so the effect imposed by PS4 is not significant.

Immunomodulatory, amphiphilic PS can be effectively complexed with negatively charged nucleic acid ligands and used as a natural targeting and delivery vehicle. When tested in culture on mouse spleen cells and RAW 264.7 mouse macrophage cell line, a strong synergism in cytokine secretion as evidenced by a panel of Th1–biased cytokines were observed (data not shown). Currently, these complexes are used as a potential delivery system aiming to test their immunoadjuvant effects in vivo.

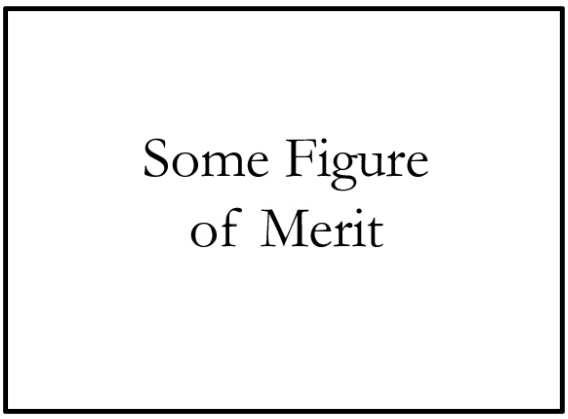

FIGURE 1. *IL-6 production of spleen cells after 42h stimulation only with PS and TLR9 ligand or PS complexed with TLR9 ligand.*

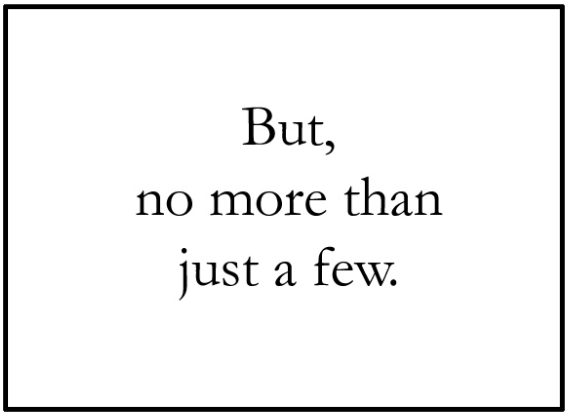

FIGURE 2*. The mRNA transcript profiles of tlr-1 to tlr-9 genes after stimulation (4h) of spleen cells with nucleic acid ligands complexed with PS4.*

ACKNOWLEDGMENTS

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REFERENCES

1. P.Balanchandran et al, Int. Immunopharm. 2006, 6 (12) : 1808