Nucleic acid polymers optimized to treat hepatitis B virus infection in patients do not harbour immune stimulatory properties in primary isolated blood or liver cells

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ABSTRACT

Background: Nucleic acid polymers (NAPs) are phosphorothioate oligonucleotides which interfere protein interactions involved in viral replication. This property is length dependent and sequence-independent. It has been demonstrated that NAPs are active against hepatitis B virus (HBV) where the antiviral mechanism is still being investigated. In this study, the immune stimulatory properties of NAPs optimized for the treatment of HBV infection *in vivo* and in the clinic were examined in primary isolated human blood and liver cells.

Methods: Human peripheral blood mononuclear cells (PBMCs) as well as primary isolated hepatocytes were treated with different concentrations of NAPs. REP2006 is a 40mer degenerate NAP $(dN)_{40}$ previously shown to have residual pro-inflammatory activity due to CpG content and REP2055 is 40mer NAP containing a sequence optimized to be devoid of CpG content $(dAdC)_{20}$ that retains antiviral activity and which has been shown to be effective *in vivo* and clinically against HBV infection. To indicate immune responsiveness, toll-like receptor ligands (polyI:C, ODN2216) were used as immune stimulatory controls. Total RNA was isolated and quantitative RT-PCR was performed to analyse gene expression of *IFNA4*, *IFNB1*, *IFNG*, *IFNL2*, *TNF*, *IL6*, and *IL10*. The intracellular uptake of CY3-labelled NAPs was visualized using fluorescence microscopy.

Results: REP 2006 induced an inflammatory cytokine response in PBMCs comparable to the ODN2216 control. However, while ODN2216 strongly induced the expression of interferons (*IFNA4*, *IFNB1*, *IFNG*, *IFNL2*), REP 2006 did not. Here just a weak signal for *IFNB1* expression could be detected when the highest concentration of 5µM was used. The treatment with REP 2055 resulted in only a weak induction of *IL6* expression in PBMC. In primary isolated human hepatocytes neither ODN2216 nor REP 2006 or REP 2055 induced the expression of any of these genes. In contrast, poly I:C-treated hepatocytes showed significant induction of *IL6*, *TNF*, *IFNB1*, *IFNG* and *IFNL2*.

Conclusion: Although some synthetic nucleic acids have been shown to be able to stimulate an innate immune response *in vivo* and *in vitro*, the data presented here demonstrate that NAPs do not induce antiviral responses in primary isolated blood or liver cells. We therefore hypothesize that the antiviral activity of NAPs cannot be explained by direct induction of innate antiviral responses.

RESULTS

ODN2216 and REP2006, but not REP2055, induce innate responses in PBMCs

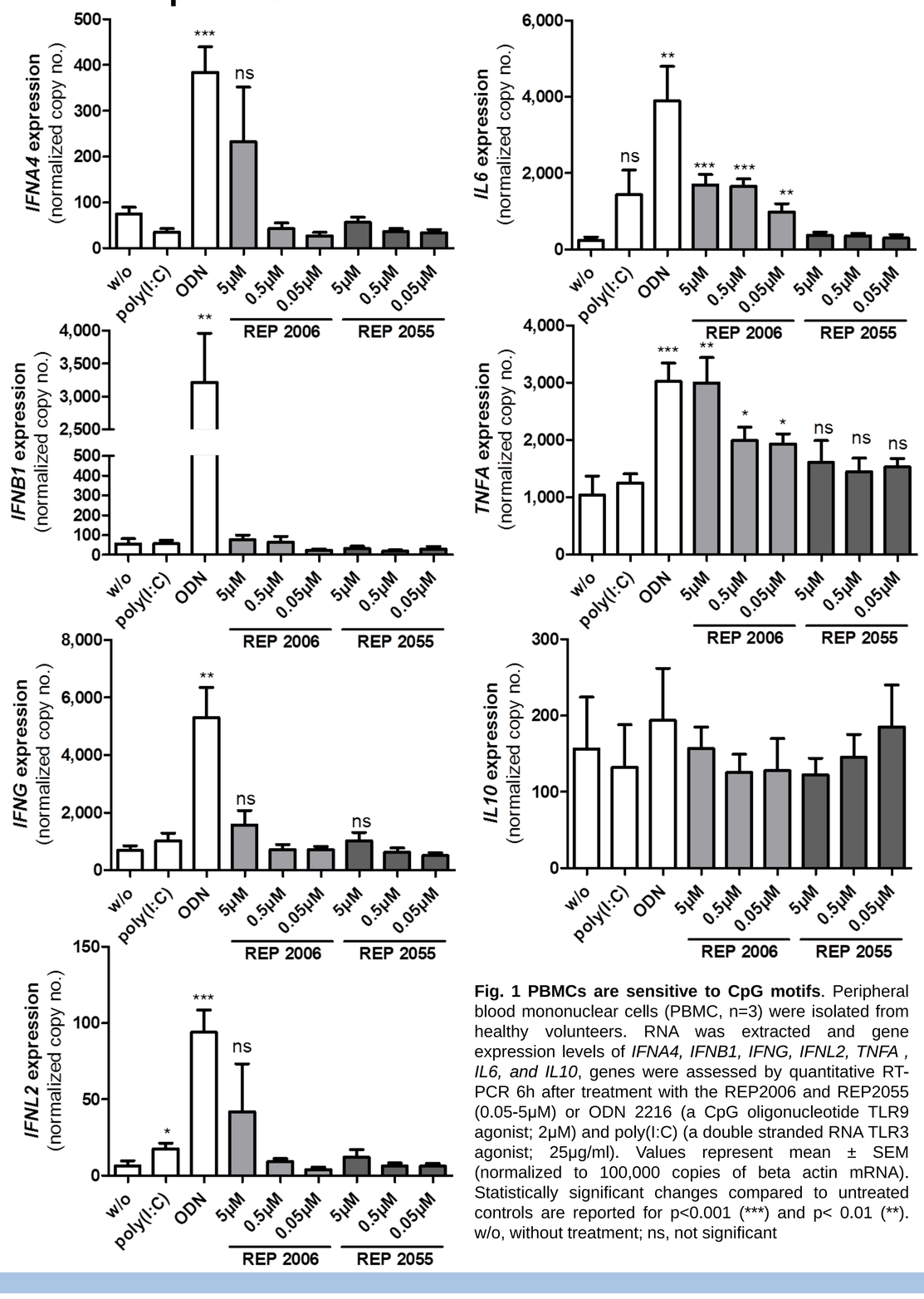

Fig. 1 PBMCs are sensitive to CpG motifs. Peripheral blood mononuclear cells (PBMC, n=3) were isolated from healthy volunteers. RNA was extracted and gene expression levels of *IFNA4, IFNB1, IFNG, IFNL2, TNFA , IL6, and IL10*, genes were assessed by quantitative RT-PCR 6h after treatment with the REP2006 and REP2055 (0.05-5µM) or ODN 2216 (a CpG oligonucleotide TLR9 agonist; 2µM) and poly(I:C) (a double stranded RNA TLR3 agonist; 25µg/ml). Values represent mean ± SEM (normalized to 100,000 copies of beta actin mRNA). Statistically significant changes compared to untreated controls are reported for p<0.001 (***) and p< 0.01 (**). w/o, without treatment; ns, not significant

Primary human Hepatocytes are not sensitive to REP2006 or REP2055

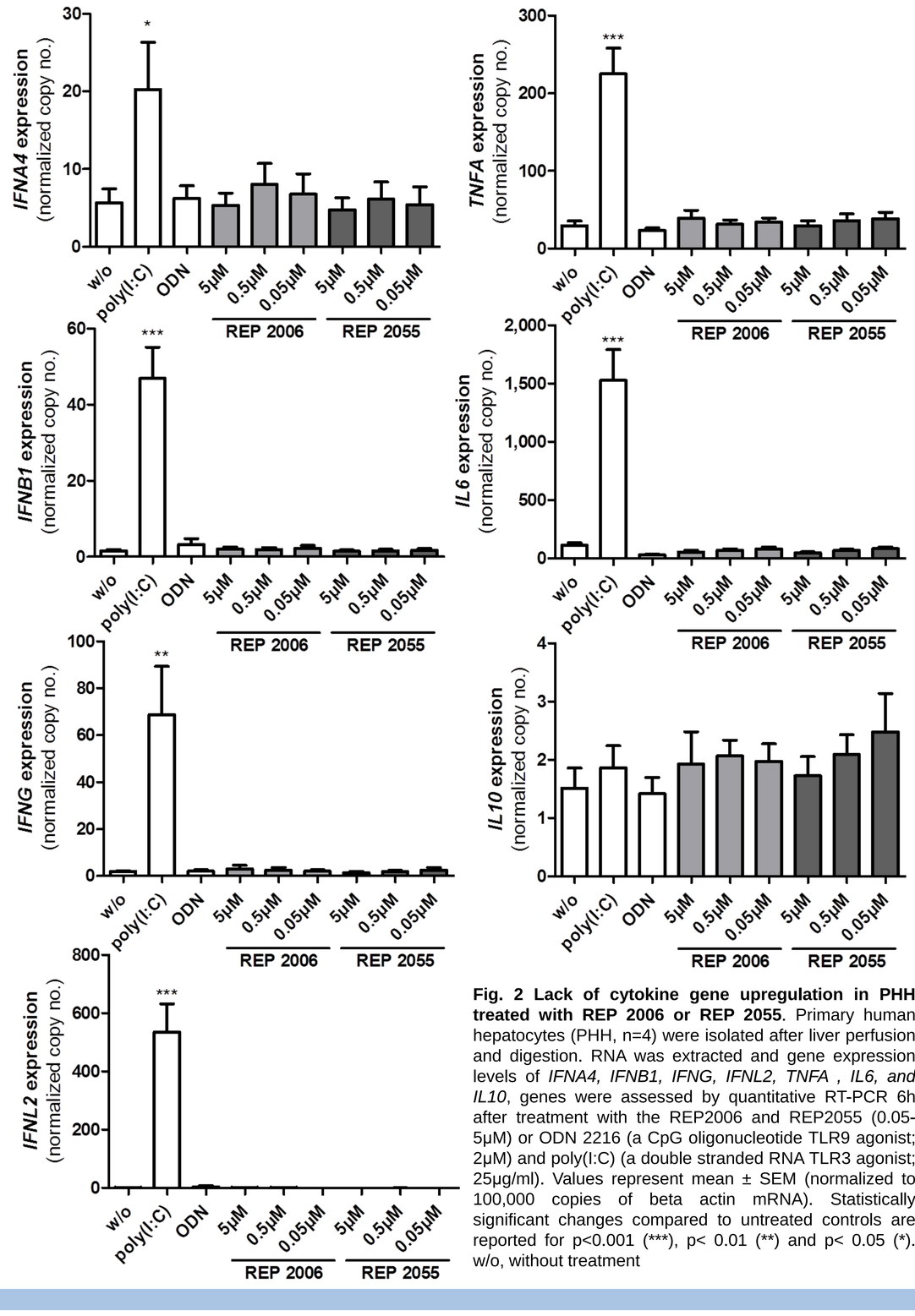

Fig. 2 Lack of cytokine gene upregulation in PHH treated with REP 2006 or REP 2055. Primary human hepatocytes (PHH, n=4) were isolated after liver perfusion and digestion. RNA was extracted and gene expression levels of *IFNA4, IFNB1, IFNG, IFNL2, TNFA , IL6, and IL10*, genes were assessed by quantitative RT-PCR 6h after treatment with the REP2006 and REP2055 (0.05-5µM) or ODN 2216 (a CpG oligonucleotide TLR9 agonist; 2µM) and poly(I:C) (a double stranded RNA TLR3 agonist; 25µg/ml). Values represent mean ± SEM (normalized to 100,000 copies of beta actin mRNA). Statistically significant changes compared to untreated controls are reported for p<0.001 (***), p< 0.01 (**) and p< 0.05 (*). w/o, without treatment

CONCLUSIONS

- **PBMCs but not PHH are highly sensitive for CpG-containing oligonucleotides**
- **REP 2006 weakly induced inflammatory cytokines in PMBCs but caused no significant interferon response in PBMCs or PHH.**
- **REP 2055 (where CpG are eliminated) did not cause any detectable pro-inflammatory or interferon response in PBMCs or PHH.**

∅ the antiviral activity of NAPs do not appear to be caused by a direct induction of innate antiviral responses.