CpG-Dependent Inflammatory Response after Delivery of Lipid/Plasmid Complexes to Murine Lungs

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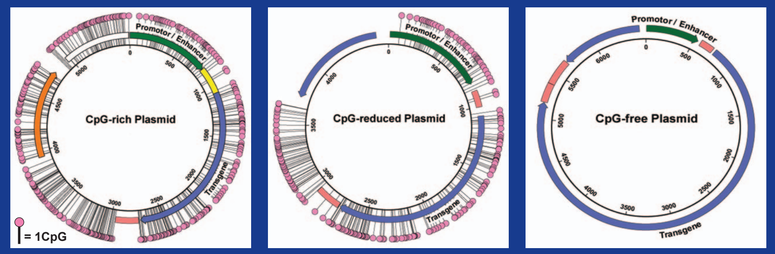
Introduction

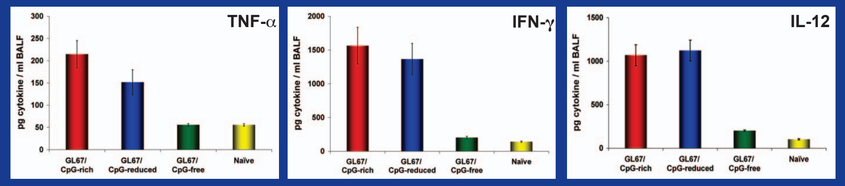
- Non-viral gene therapy is being developed for the treatment of Cystic Fibrosis (CF) lung disease
- Pre-clinical and clinical studies have shown an inflammatory response after lung delivery of the cationic lipid GL67 complexed with plasmid DNA
- Bacterially derived, unmethylated CG dinucleotide (CpG) motifs present in plasmid DNA have been implicated in the inflammatory response
- A marked elevation in the pro-inflammatory cytokines TNF-α, IFN-γ, and IL-12 following lung delivery has been attributed to the CpG response
- We have searched for additional inflammatory markers following delivery of GL67/plasmid complexes to the mouse lung

Plasmid expression vector constructs



- CpG-reduced plasmid has 40 % fewer CpGs than CpG-rich plasmid
- 80 µg plasmid was complexed with cationic lipid GL67
- 100 ml GL67/plasmid complex was delivered by intranasal instillation to the lungs of female BALB/c mice (n = 6 to 10 per group)
- 24 hours post dosing lung lavage fluid (3 ml) was recovered and assayed by ELISA for TNF-α, IFN-γ, and IL-12 or by Bio-Plex for 23 cytokines

Host inflammatory response upon delivery of GL67/ plasmid complexes to the mouse lung

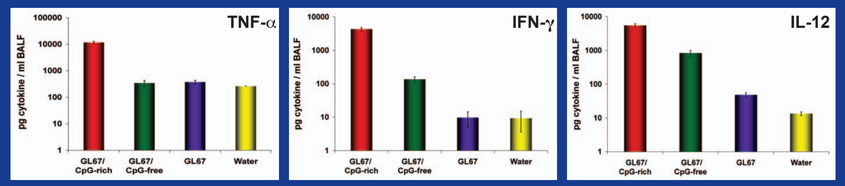


- Delivery of GL67/CpG-rich plasmid complexes resulted in increased TNF-α, IFN-γ, and IL-12 ($p < 0.0001$, ANOVA/PLSD)
- A 40 % reduction of CpG motifs did not lead to a significant decrease in IFN-γ and IL-12 ($p > 0.05$), and resulted in only a small significant decrease in TNF-α compared with CpG-rich complexes ($p = 0.0357$)
- Delivery of GL67/CpG-free plasmid complexes substantially reduced cytokine levels compared with CpG-rich complexes ($p < 0.0001$)

The 23 cytokines measured using Bio-Plex

TNF-α	IL12p70	KC	MIP-1α	IL-5	IL-9	IL-2	GM-CSF
IFN-γ	IL-6	G-CSF	MIP-1β	IL-10	IL-1β	IL-3	Eotaxin
IL-12p40	RANTES	MCP-1	IL-1α	IL-13	IL-17	IL-4	

- Standard marker cytokines for a CpG inflammatory response are shown in red



- Results with the three standard cytokines measured previously by ELISA were confirmed using the Bio-Plex method
- Delivery of GL67/CpG-rich plasmid complexes significantly increases cytokine levels ($p < 0.0001$)
- GL67/CpG-free plasmid complex delivery resulted in a reduced cytokine response compared with CpG-rich complexes ($p < 0.0001$), but levels of IFN-γ and IL-12 were still higher compared with water treated animals ($p < 0.0001$)

A significant increase in 13 cytokines was seen compared with water treated animals

Cytokine	GL67/CpG-rich fold increase	GL67/CpG-free fold increase	GL67 fold increase
TNF-α	44.8	ns	ns
IFN-γ	480.0	15.1	ns
IL-12p40	424.5	64.3	3.7
IL12p70	103.9	2.0	2.8
IL-6	323.0	45.6	2.8
RANTES	182.0	34.5	ns
KC	21.1	23.1	12.4
G-CSF	309.9	33.3	74.1
MCP-1	42.7	9.5	ns
MIP-1α	5.7	2.2	ns
MIP-1β	53.9	7.8	3.4
IL-1α	11.9	2.7	4.5
IL-5	15.5	7.6	5.9

- 13 out of 23 cytokines tested showed a highly significant increase after delivery of GL67/CpG-rich plasmid complexes ($p < 0.0001$)
- Delivery of GL67/CpG-free plasmid complexes resulted in significant increases in 12 cytokines ($p < 0.05$), but the magnitude was smaller compared with CpG-rich complexes (ns = non significant)

Conclusions

- A high-throughput approach for measuring cytokine responses has been used successfully in the mouse lung
- Following delivery of lipid/plasmid DNA complexes, 10 cytokines showed significant elevation in addition to the standard TNF-α, IFN-γ, and IL-12 markers
- Lipid complexed with CpG-free plasmid DNA showed a reduced pro-inflammatory cytokine profile, compared with CpG-rich plasmid complexes
- Lipid alone generated a small inflammatory response, though not as strong as when complexed with plasmid DNA

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