CHDR Centre for Human Drug Research

Characterization of inflammation and immune cell modulation induced by low-dose LPS administration to healthy volunteers

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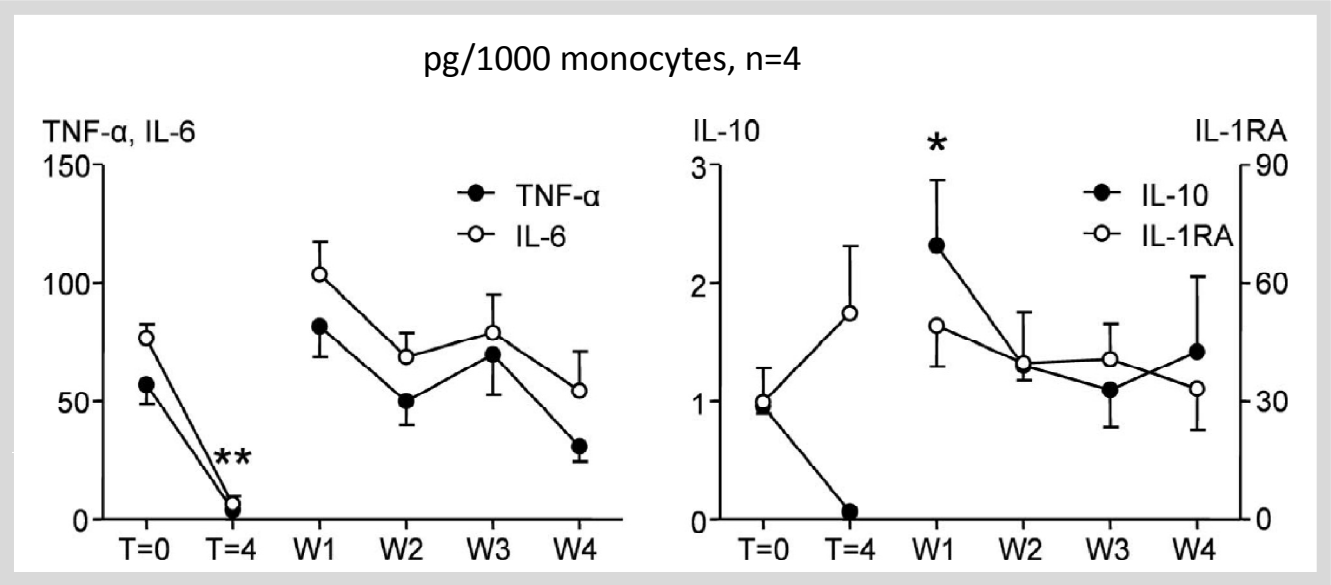
Human endotoxin model – introduction

- A model of systemic inflammation
  - Flu-like symptoms
  - ↑ CRP production
  - ↑ Concentrations pro- and anti-inflammatory cytokines
- Administration of purified LPS (endotoxin) from *E. coli* or other Gram-negative bacteria
- *E. coli*: high reproducibility of effects *(Andreasen et al.)*
- High LPS doses, not preferred
  - Potential effects of immune-modulating interventions might not be observed
  - Not free of risk for the volunteer (a.o. cardiovascular)
  - Other homeostatic mechanisms may be temporarily impaired

LPS hyporesponsiveness

- Follows upon *in vivo* LPS challenge
  - Altered cytokine production
  - ↓ Inflammatory response following LPS rechallenge
- Many negative regulators (e.g. SOCS-1, IRAK-M, and SHIP) of the TLR4 signaling pathway *(Fu et al. 2012, Morris et al. 2011)*

LPS hyporesponsiveness – *Kox et al. 2011*



- T=0: *in vivo* challenge
- T=4hrs and 4 wks: *ex vivo* challenge

- *Ex vivo* LPS hyporesponsiveness
  - Resolved 1 week after *in vivo* LPS challenge
  - Exact time course unclear
  - Possible differences between read-outs

Study objectives

- To assess the relationship between administration of low doses of LPS (0.5, 1 and 2 ng/kg) and the inflammatory response (cytokine levels and CRP) in healthy male volunteers

- To assess the duration of hyporesponsiveness of the immune system after *in vivo* LPS administration, as determined by *ex vivo* LPS challenges

Study outline (1)

- Randomized, blinded, placebo controlled, sequential-group study
- 24 healthy male subjects
  - 3 cohorts (active-pl: 6-2)
- Ascending single iv doses of 0.5-2ng/kg LPS
  - U.S. Reference *E. Coli* endotoxin Lot#3 (O113:H, 10:K negative, ~10IE/ng)
- IV hydration
  - Pre-hydration 2hrs pre-dose: 1500mL glucose/saline
  - Hydration 6hrs post-dose: 150mL/hr

Study outline (2)

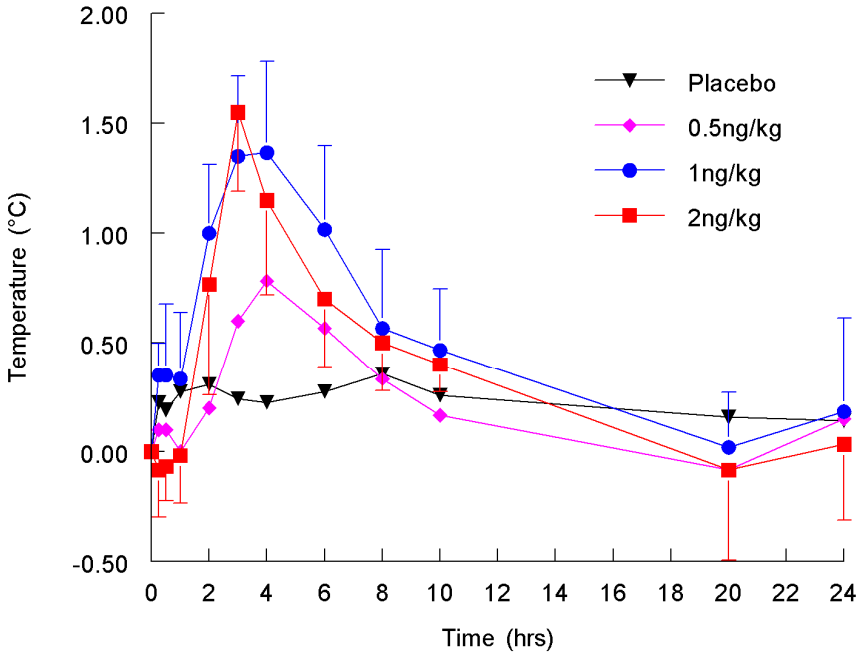
- Inflammatory response, *in vivo*
  - CRP
  - IL-1β, IL-6, IL-8 and TNF-α (human 4-plex, MSD)
- Inflammatory response, *ex vivo*
  - Whole blood cultures with LPS (*E. Coli*, O111:B4, ~10IE/ng)
    - 24 hrs incubation at 37° C, 5% CO_2
  - -2hrs, 6, 12, 24, 48 and 72hrs
  - IL-1β, IL-6, IL-8 and TNF-α (human 4-plex, MSD)
- Safety
  - AEs / vital signs / ECG / routine labs

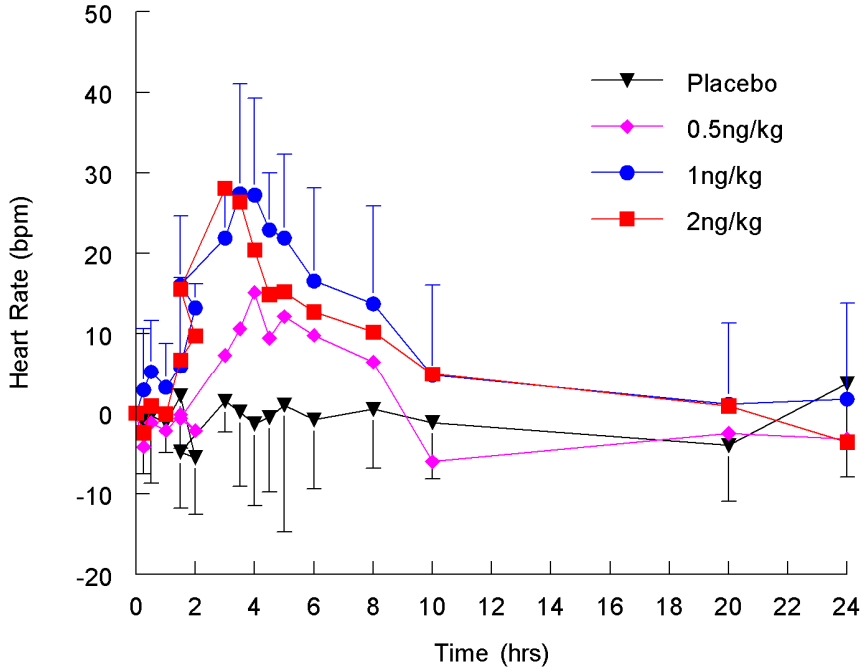
Study results – Safety

- Well tolerated, no clinically relevant changes or unexpected treatment-related trends in
  - Urinary or blood laboratory parameters
  - ECG recordings
  - Vital signs
  - AEs: mild severity and self-limiting

- Most frequent reported AEs
  - Headache; 66.7% of the LPS-treated subjects, 33.3% of the placebo-treated subjects
  - Feeling cold; 44.4% of the LPS-treated subjects, none of the placebo-treated subjects

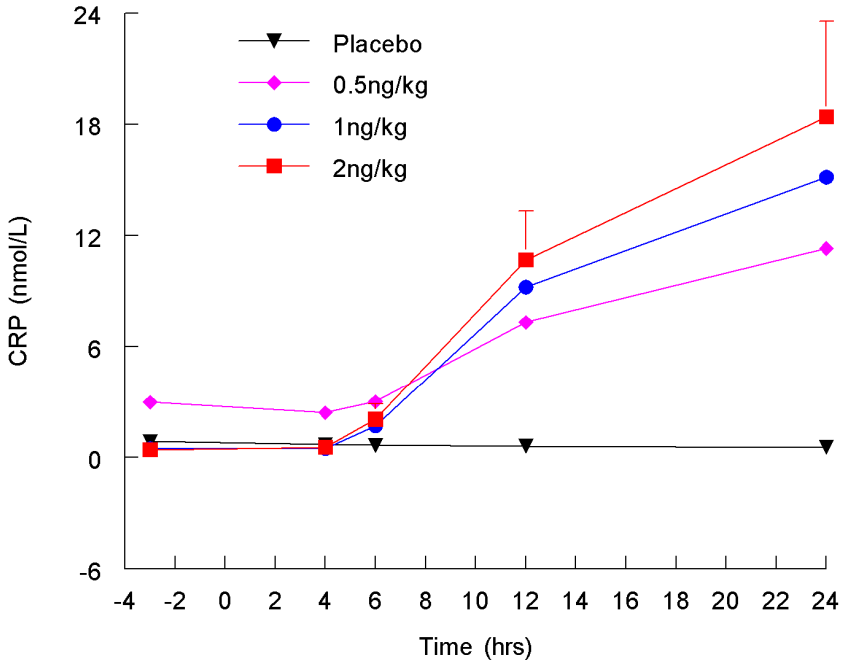
Study results – Safety – Temperature and HR





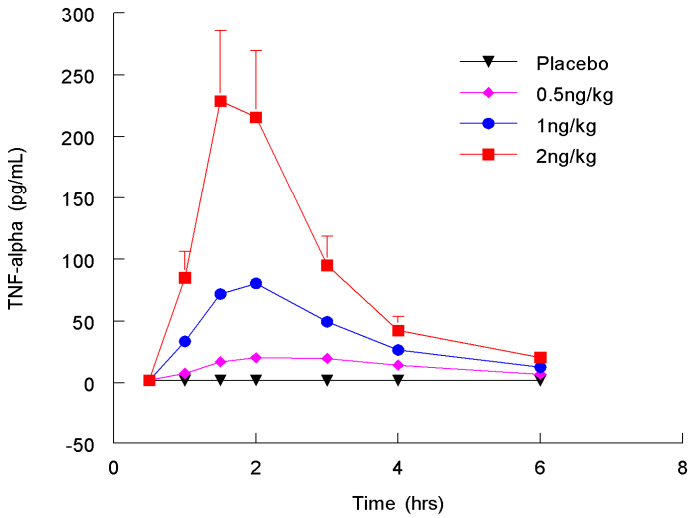
- BP highly variable over time, max. mean decreases in the range of 0 to -13 mmHg

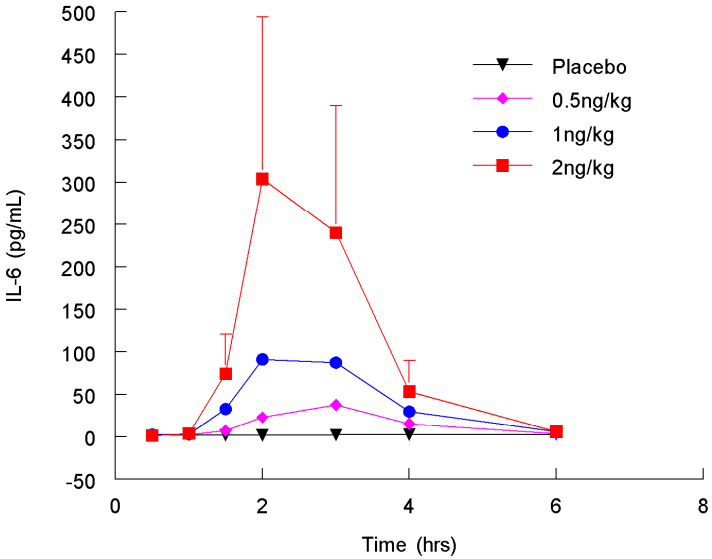
Study results – *In vivo* CRP

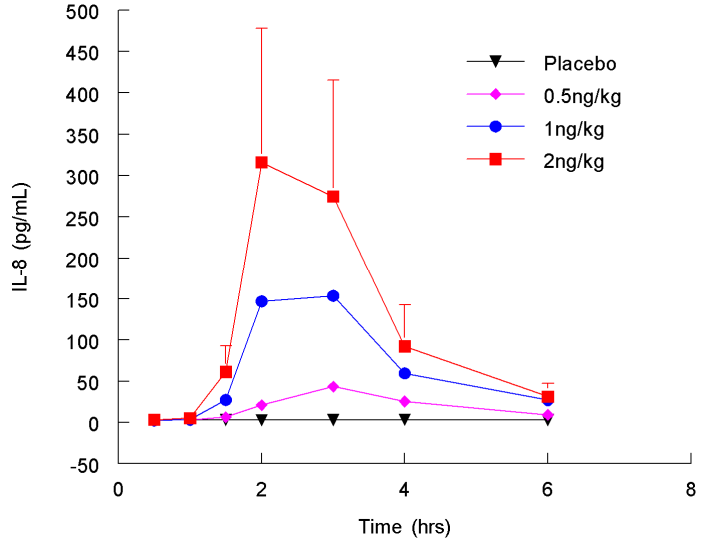


- Statistical analysis
  - Significant contrasts, dose groups (0.5, 1, 2ng/kg) vs pl: $p<0.0001$

Study results – Circulating cytokines (TNF-α, IL-6, IL-8)

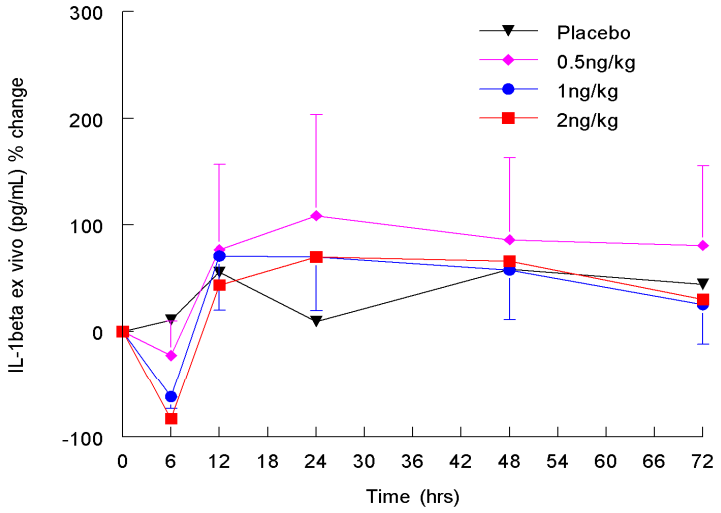


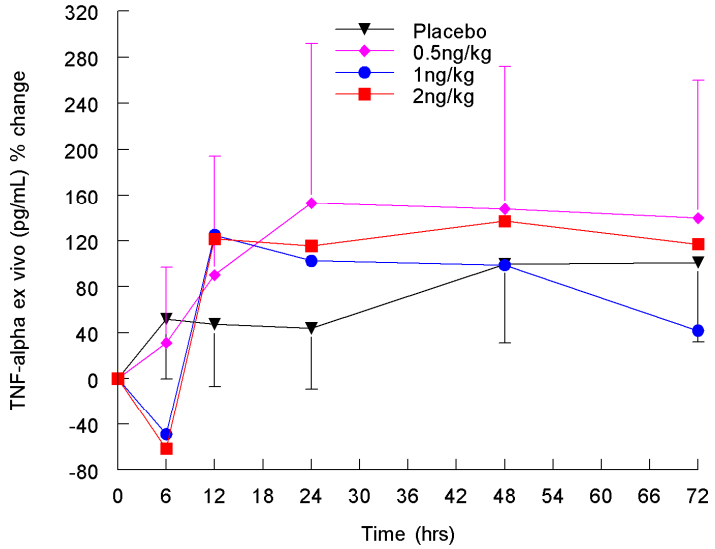




- T_{max}: 1.5-3hrs post-dose
- Statistical analysis
  - Significant contrasts up to t=6hrs: (0.5, 1, 2ng/kg) vs pl: $p<0.0001$
- IL-1β: ↑ 3-6hrs post-dose (2ng/kg)

Study results – LPS Hyporesponsiveness

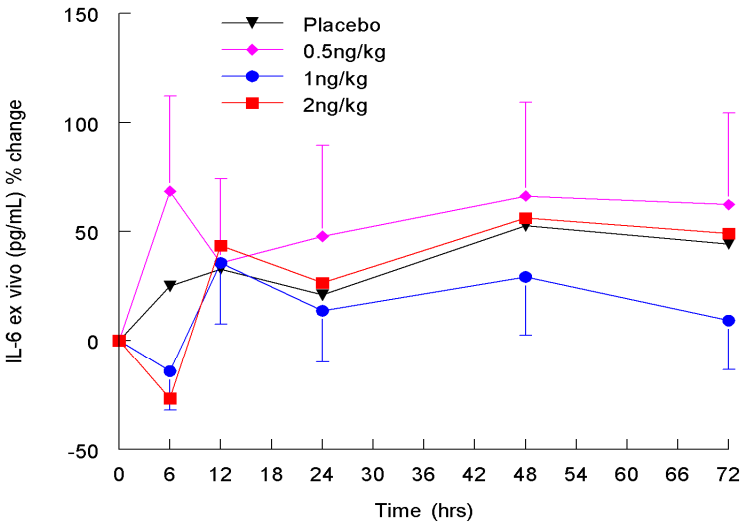


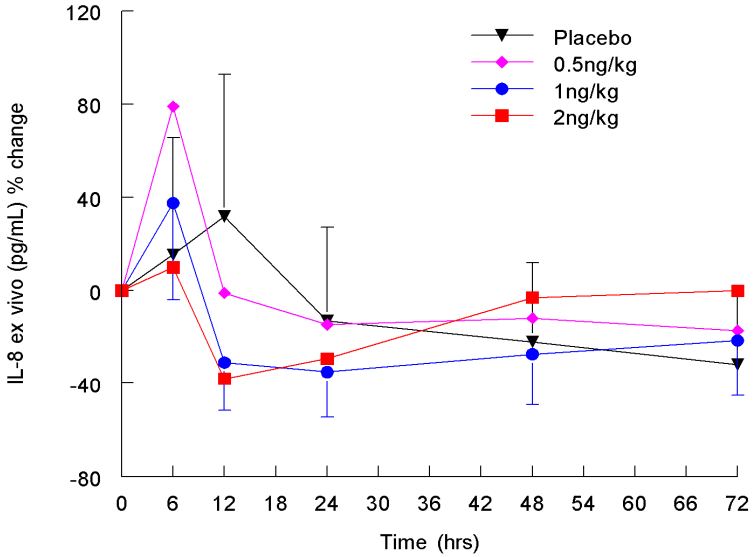


- Statistical analysis

	Contrast at 6hrs versus pl	Estimated difference (%)	p-value
IL-1β	1ng/kg	-65.8	<0.0001
	2ng/kg	-84.7	<0.0001
TNF-α	1ng/kg	-66.4	0.0005
	2ng/kg	-74.7	<0.0001

Study results – LPS Hyporesponsiveness





- Statistical analysis

	Contrast at t=6hrs vs pl	Estimated difference (%)	p-value
IL-6	1ng/kg	-31.3	0.0283
	2ng/kg	-41.3	0.0024
IL-8	0.5ng/kg	55.1	0.0879
	1ng/kg	19.2	0.4961
	2ng/kg	-4.8	0.8475

- [IL-8] and [IL-6] response ≠ [TNF-α] and [IL-1β]: indication for priming?

Conclusions

- LPS doses 0.5-2ng/kg: well-tolerated
- PD parameters: cytokine release and safety markers (temperature and heart rate)
- LPS doses ≥ 0.5ng/kg: distinct inflammatory response
- LPS dose-dependent hyporesponsiveness observed for IL-1β, IL-6 and TNF-α after *ex vivo* LPS stimulation:
  - Max. measured 6hrs post-dose
  - Total duration of ~12hrs
- Clinical pharmacology studies: application of a combination of *in vivo* LPS administration and repeated *ex vivo* LPS challenges

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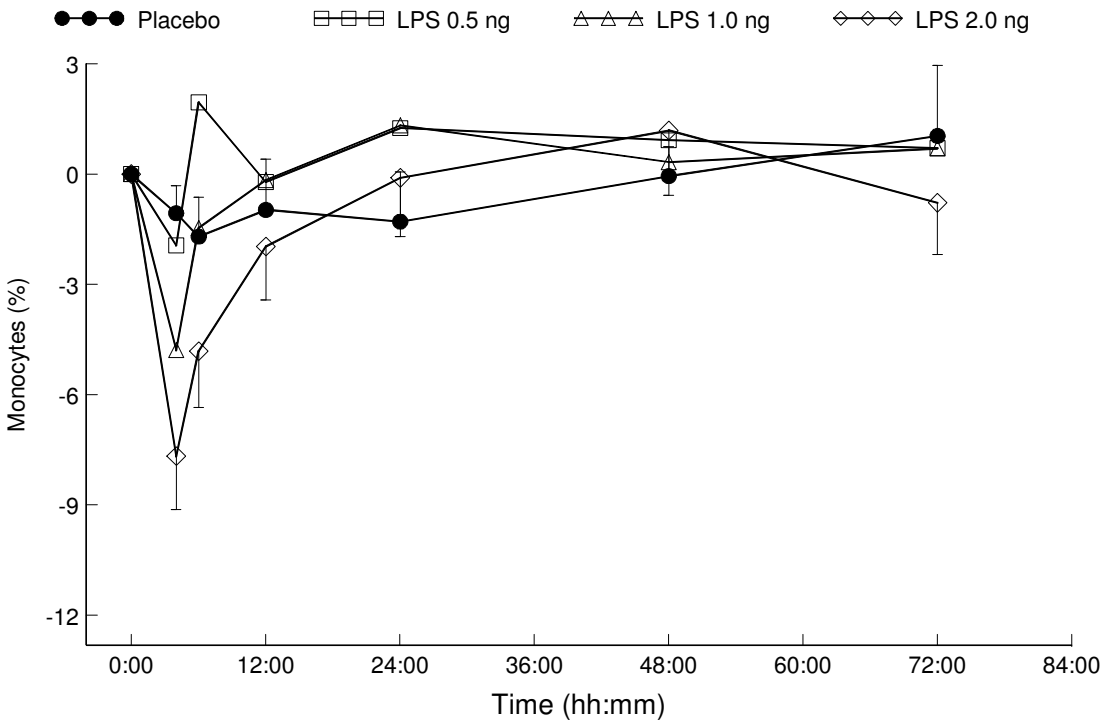
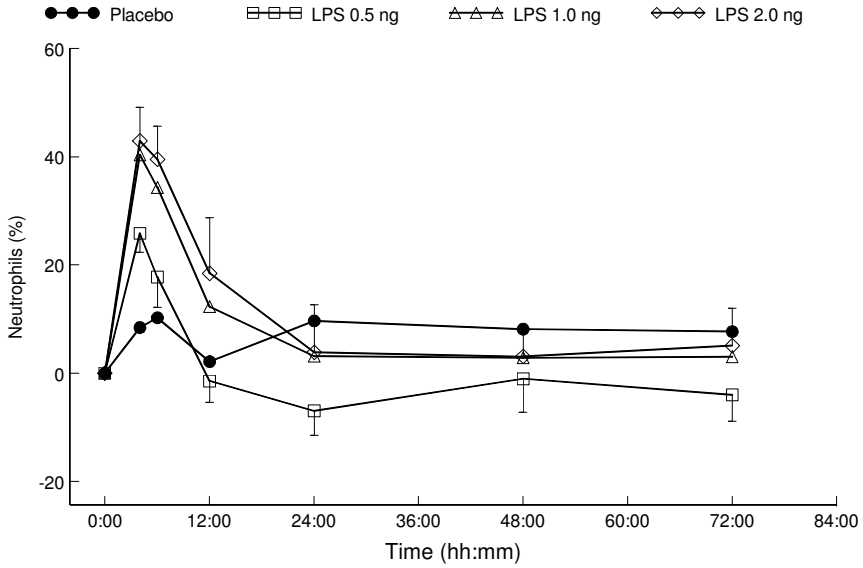
unlocking the true potential

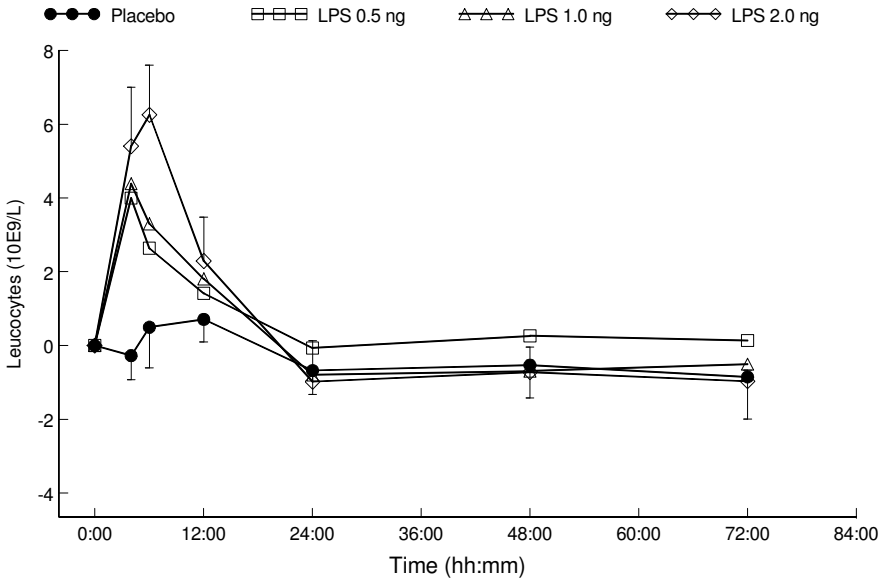
Study results – Safety – Monocytes



- Monocyte count: dose-dependent decrease with a minimum change from baseline at 6hrs post-dose, returning to baseline 12-24hrs post-dose

Study results – Safety – Neutrophils / Leucocytes

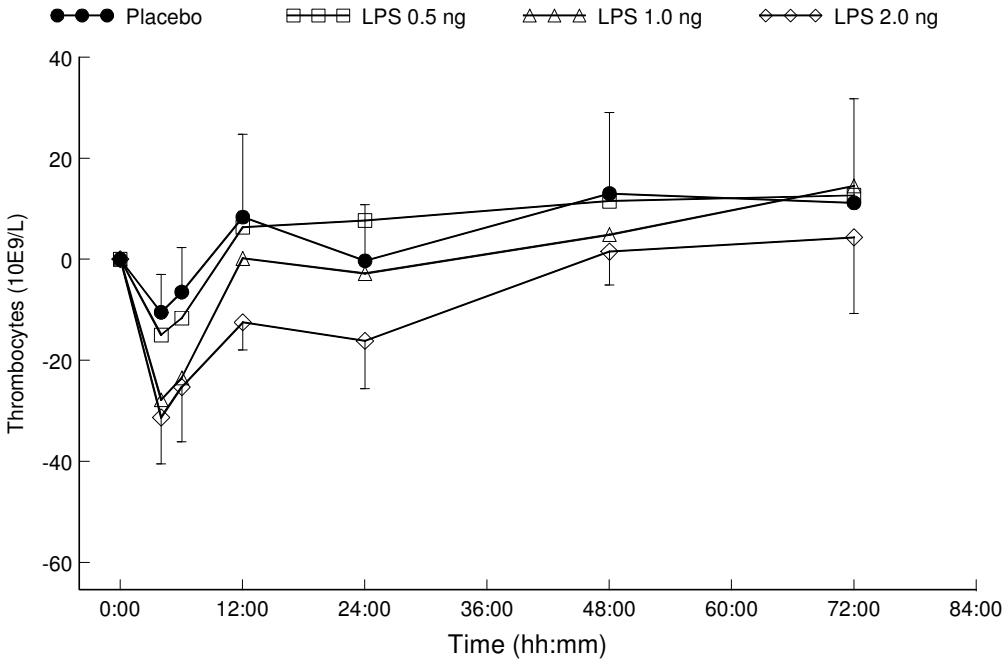




- Neutrophil count: peak levels at 4hrs post-dose
- Leucocyte count: peak levels at 4-6hrs post-dose

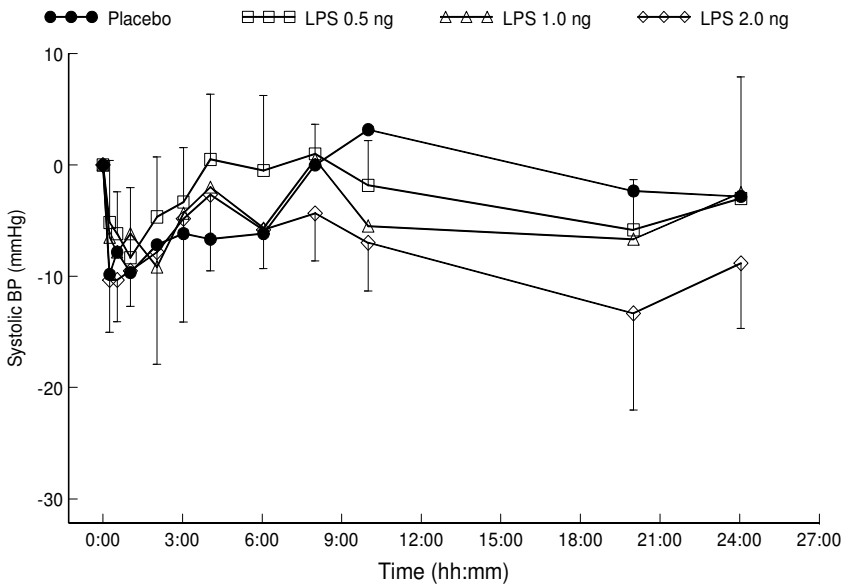
} returning to baseline 12-24hrs post-dose

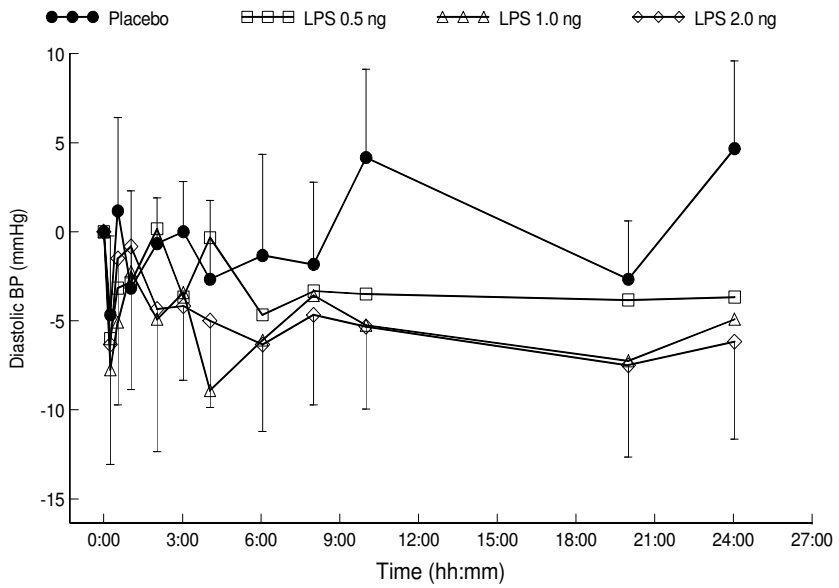
Study results – Safety – Thrombocytes



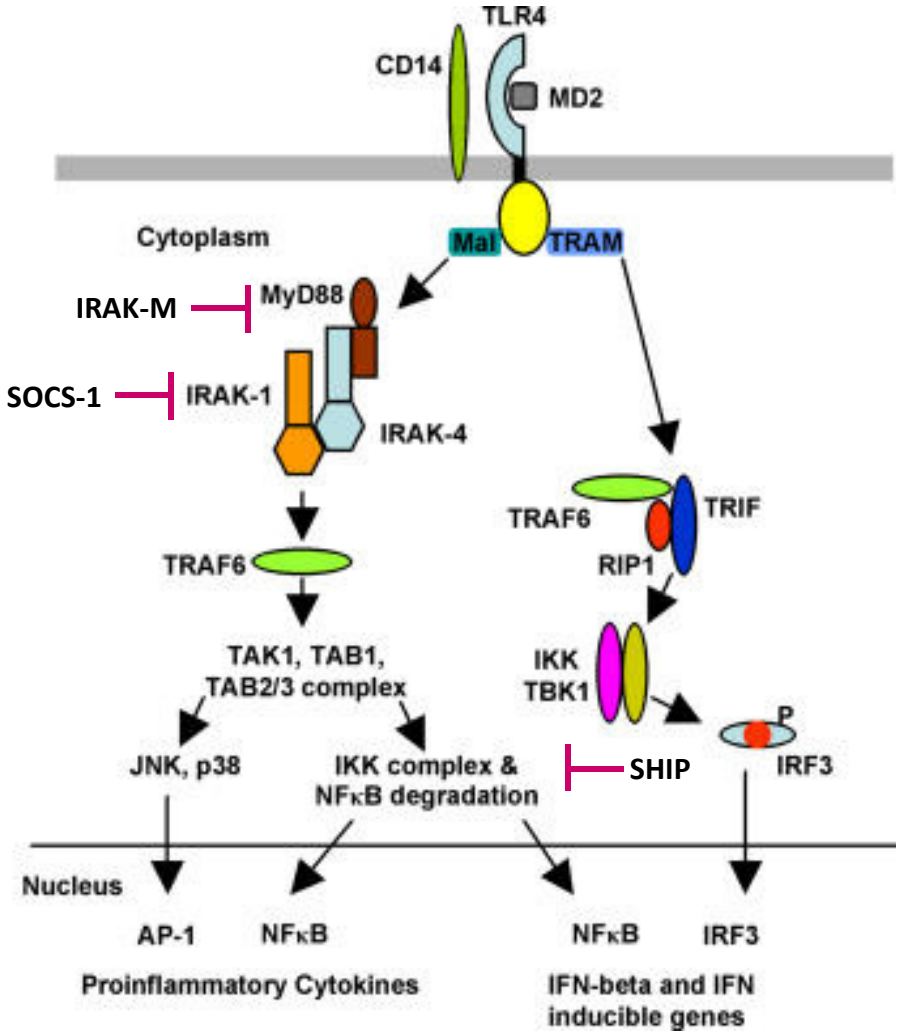
- Blood platelet count (thrombocytes): dose-dependent decrease with min. mean levels ~4hrs post-dose, returning to baseline levels around 12 (0.5 and 1ng/kg) and 48hrs post-dose (2ng/kg)

Study results – Safety – BP





LPS signaling pathways



Human endotoxin model – Priming

- Follows upon *in vivo* LPS challenge with very low doses
  - ↑ Inflammatory response following LPS rechallenge
  - May enable the immune system to elicit a strong inflammatory response against potential pathogens *(Fu et al.)*

- Priming in animals described extensively, but underlying mechanisms poorly understood

Power calculation (TNF-α, IL-6 and CRP)

- Parallel study design, LPS dose level: 0.5 ng/kg, sample size of 8 subjects per treatment group
  - 80% power to detect (two-sided significance level of 0.05)
    - 28% inhibition in the LPS-induced TNF-α response;
    - 53% inhibition in the LPS-induced IL-6 response;
    - 49% inhibition in the LPS-induced CRP response.

- Inter subject variability on log scale is well comparable between different LPS doses - this power calculation also applies for LPS doses of 1 and 2 ng/kg