TRANSIENT SUPPRESSION OF PRO-INFLAMMATORY T-CELL SUBSETS AFTER LPS CHALLENGE IN A HUMAN, EXPERIMENTAL ENDOTOXINAEMIA MODEL

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Introduction:

- Systemic inflammatory response syndrome (SIRS) is a multifactorial inflammatory response of an organism to infection or endotoxins, e.g. lipopolysaccharides (LPS). Immune responses, with predominant pro- and anti-inflammatory properties, can be observed in SIRS.
- Clinical features of SIRS: Fever/ Hypothermia, elevation of breathing- und heart frequence and a leukocytosis/ leukopenia.
- T-cells provide pro- and anti-inflammatory functions. Regulatory T-cells (Tregs) have a piovital role in immune regulatory processes amongst others via production of Interleukin 10 (IL-10), which is the most important anti-inflammatory cytokine.
- Our study has the aim to investigate the T-cell response in an experimentally induced, human endotoxinaemia model, classical induced by LPS challenge.

Methods and volunteers:

- Randomisied and placebo-controlled Crossover-Study (**Fig.1**).
- 20 healthy men (26.1 ±4,2 [range: 18-34] years).
- Continous controll of vital signs for provement of SIRS criteria.
- LPS (*Eschericha coli* LPS) low-dose of 0,8 ng/kg BW or a placebo (NaCl, 0.9%) was administrated intravenously with a 72h surveillance of cellular immune responses.
- FACS: Phenotyping of Tregs ($CD4^+/CD25^{hi}/CD127^{low}$,[**Fig.2**]), IFACS of IL-10 [**Fig.3**], IFNy [**Fig.6**], IL-2 and IL-17A [both not depicted]) and the quantification of $CD3^+/4^+/8^+$T-cells (**Fig.7**) were performed.
- Systemic cytokine analysis of IL-10 was done by ELISA (**Fig. 5**).
- Bonferroni-corrected paired T-Tests was performed: *p<.05, **p<.01, ***p<.001. (*) show significant results before Bonforroni-correction.

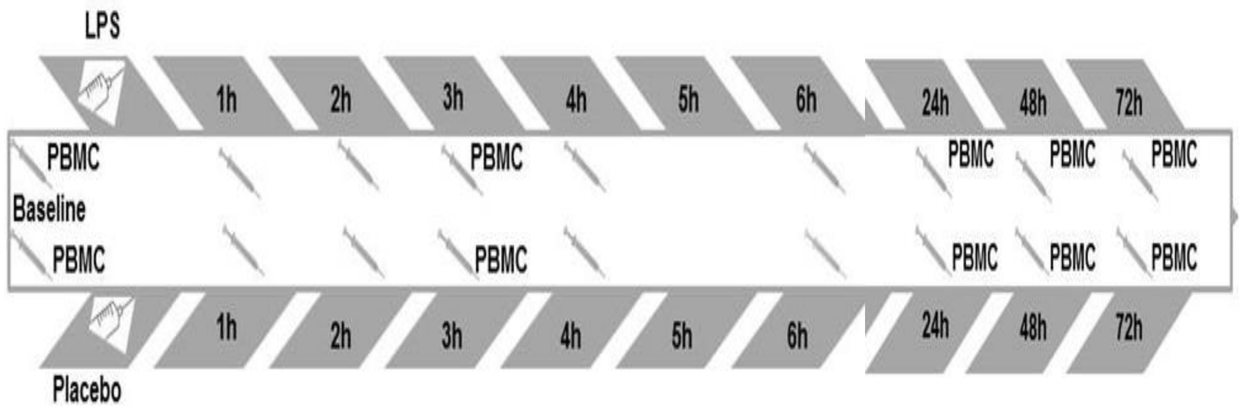

Fig.1:Study design. Baseline: time point before placebo/LPS application. PBMC: isolation of peripheral blood mononuclear cells for T-cell assays with surveillance over 72h . Grey syringe: vital sign controll and blood taking for complete blood counts, PBMC isolation, cellular and cytokine analyses.

Results:

- SIRS criteria showed 3 hours after LPS application: hyperthermia/fever (p< .0001), elevation of heart- (p< .0001) and breathing frequence (p= .007) and a leukocytosis (p< .0001, SIRS criteria not depicted).
- Relative Treg kinetics were stable after LPS- and placebo application over 72h (**Fig.2**), even in $CD4^+IL10^+$ T-cell compartment (**Fig.3**).
- Systemic IL-10 concentration showed a significant elevation (**Fig. 5**).after LPS administration with a maximum after +2h post injectionem (p.i.).
- Pro-inflammatory T-cell subsets e.g. $CD4^+IFNy^+$ T-cells (**Fig.6**) and $CD3^+$ /$CD4^+/8^+$ T-cells (**Fig.7**) showed a cell nadir 3h after LPS administration.

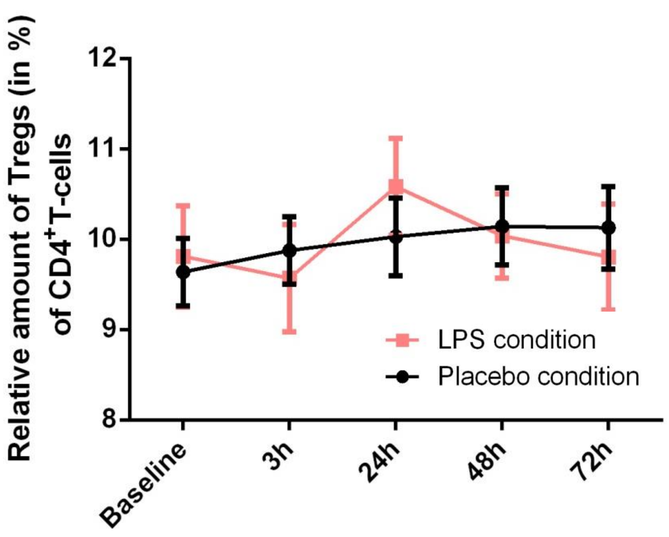

Fig.2: Relative Treg compartment of CD4+T-cells (in %) after LPS- (red depicted) and placebo administration (black depicted).

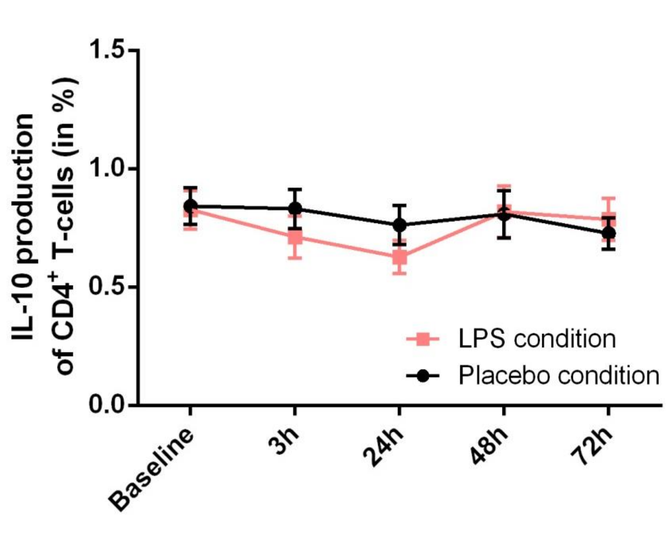

Fig.3: Anti-inflammatory T-cell subset: $CD4^+IL10^+$ T-cells (in %) after LPS- (red depicted) and placebo administration (black depicted).

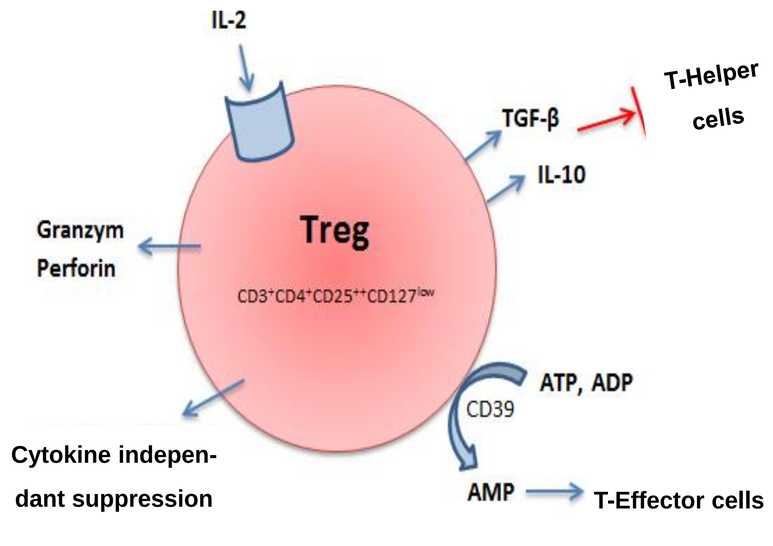

Fig.4: **Regulatory T-cell** (Treg) and ist function during inflammation as a regulator of immune response.

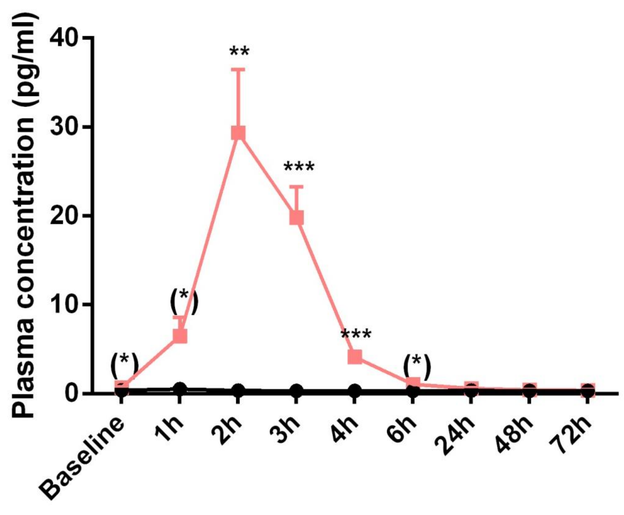

Fig.5: Systemic IL-10 concentration after LPS-application with a maximum 2h after LPS - (red depicted) vs. Placebo administration (black depicted).

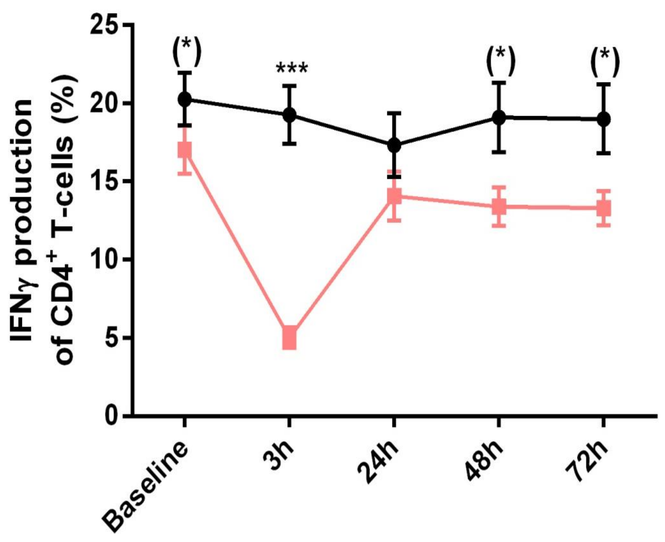

Fig.6: Pro-inflammatory T-cell subset: $CD4^+IFNy^+$ T-cells (in %) after LPS- (red depicted) and placebo administration (black depicted).

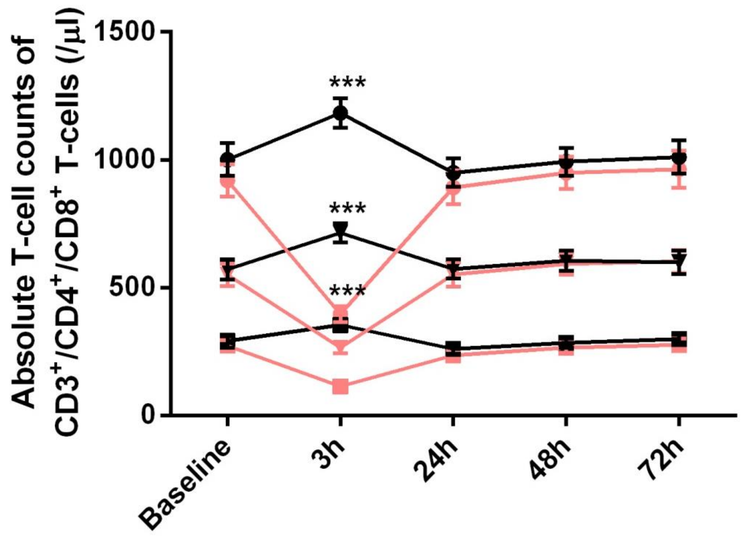

Fig. 7: **Quantification of $CD3^+$** (top set of curves), **$CD4^+$**(mid-set of curves) and **$CD8^+$**T-cells (lowest set of curves) under LPS (grey lines) and placebo (black lines) condition with significant decreases of pro-inflammatory T-cell subsets after 3h after LPS administration.

Conclusion:

- **The circulating pro-inflammatory T-cell compartments diminished transiently after LPS application most likely due to the increase of IL-10**
- **The IL-10 level sharply increased potentially secreted by highly activated $Tregs^{IL\text{-}10+}$ (Fig. 4)**
- **IL-10 may have a pivotal role in immunoparalysis in systemic inflammation by suppression of pro-inflammatory T-cell subsets**

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