INTRACELULAR GROWTH CONTROL OF *MYCOBACTERIUM TUBERCULOSIS* WITH CYTOKINES EXPRESSED IN SPLEEN CELLS STIMULATED WITH DSE

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Purpose of the study: analyze if supernatants from murine spleens stimulated with *M. tuberculosis* specific murine Dialyzable Spleen Extract (DSE^{TB}) are capable of controlling intracellular growth of *M. tuberculosis* inside J774A.1.

Methods: DSE^{TB} obtention. *M. tuberculosis* H37Rv immunized Balb/c spleens were disrupted into single cell suspensions and disrupted by freeze-thaw cycles, the extract were dialyzed against water and the dialyzate freeze-dried (1Unit of DSE^{TB} = $1x10^6$ cells). **Spleen supernatants stimulated with DSE^{TB}.** Suspensions from one healthy BALB/c spleen were incubated for 24h/37 C/5% CO_2 with 0.1 U DSE^{TB}, cell free supernatants were collected and filtered. **Activation of infected J774A.1.** J774 monolayers were incubated either before or after *M. tuberculosis* H37Rv infection (MOI 1:10) with concentrated or serial dilutions of DSE^{TB} spleen stimulated supernatans. CFU were assessed at 3, 24 and 48h. **Real time RT-PCR**. IFN gamma, IL-12p40 and IL-2 mRNA expression was measured in DSE^{TB} stimulated spleen cells.

Results: J774A.1 macrophages incubated with DSE^{TB} activated spleen supernatants (1:4) showed an statistical significant reduction in CFU at all time points ($p \leq 0.001$ Tukey). Regardless if supernatant was added before or after infection. Murine splenocytes stimulated with DSE^{TB} showed a two fold increased for IFNγ mRNA at 2 h, and a reduction of IL-12 mRNA from 2 to 24 h after stimulation.

Conclusion: Intracellular multiplication of *M. tuberculosis* H37Rv was controlled with DSE^{TB} activated spleen cells supernatants. DSE^{TB} stimulated spleen cells for cytokine production and these in turn induced an activation state in the macrophages which allowed intracellular control multiplication of the mycobacteria.