[PS1.4]

Single Oral Gavage with Live *E. coli* Stimulates Early Systemic TNF-alpha and Nitric Oxide Production in Mice

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Introduction: Early systemic increase in nitric oxide (NO) production by inducible nitric oxide synthase (iNOS) after single oral inoculation of mice with *Escherichia coli* was previously reported[1] and possible causes for it are investigated further.

Methods: Twenty-four female SPF BALB/c mice were orally inoculated with 10^8 CFU *E.coli* ATCC 25922 (in 0.1ml PBS) and euthanized 2.5h, 7h, 13h and 25h post inoculation (6 mice at each time interval). The levels of organ (lungs, liver, kidney and brain) NO and plasma endotoxin, TNF-alpha and nitrite/nitrate (NOx) were compared to those found in sham-inoculated (0.1ml PBS p.o.) mice (6 mice per time interval). Organ bacterial culture with biochemical identification and pulsed field gel electrophoresis typization of *E.coli* isolated and immunohistochemistry for iNOS was performed on lungs, liver, kidneys and brain from all mice.

Results: Organ NO and plasma TNF-alpha levels were higher in *E.coli*-inoculated animals, reaching statistical significance in all four tested organs at 7h and 25h (NO) and at 2.5h (TNF-alpha) after inoculation, in comparison to sham-inoculated animals. No differences were detected in plasma endotoxin levels, NOx or iNOS immunostaining for any of the animal groups. *E.coli* ATCC 25922 was only isolated from the lungs of one mouse 7h after inoculation.

Discussion: Single oral gavage with live *E.coli* stimulates a systemic immune response in mice as determined by plasma TNF-alpha and production of NO in the major internal organs, although organ iNOS is likely not the main source of NO produced[2]. As no bacteremia and endotoxemia were observed, the stimulus for TNF-alpha/NO production should be investigated further; especially the role of bound-endotoxin (i.e. LPS-LBP complexes)[3] and free-soluble surface material from *E.coli*[4]. In contrast to *P. gingivalis* studies[5], plasma NOx levels do not reflect organ NO production in oral *E.coli* infections[6], suggestive of different NO metabolic pathways stimulation by different Gram-negative bacteria[7-9].

References

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