Bacteria-specific biomarkers in mouse-models of infections investigated by NMR spectroscopy

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Introduction: In an attempt to develop new methods for early diagnosis of bacterial infections the metabolomics approach has already been applied to growing cultures [1], for the differentiation between bacterial and viral meningitis [2] as well as for different bacterial lung infections [3]. Here we have used metabolomics to try to understand the pathological mechanism of infections leading to sepsis. In mouse models of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* infections, sera were analyzed and by comparing their metabolomic profiles with footprints of bacterial cultures potential bacteria-specific biomarkers could be identified. It is well known that the immune cascade that is activated in response to the lipopolysaccharides (LPS) from the cell wall of gram negative bacteria mainly acts through the Toll-like receptor 4 (TLR4) [4]. However, the influence of further bacterial constituents is not yet disclosed. Therefore we also compared serum metabolite changes resulting from LPS treatment and from *E. coli* infection in both wild-type and TLR4 deficient mice.

Experiments: *NMR-based metabolic profiling:* For NMR profiling, one-dimensional nuclear overhauser enhancement spectroscopy (NOESY) spectra were acquired on a 600 MHz Bruker Avance NMR spectrometer with a 5 mm TXI probe head equipped with z-gradient. The metabolomic profiles were created using targeted profiling in Chenomx NMR Suite 4.6 [5, 6]. After normalizing the data to minimize the diurnal variation, the profiles were used as input in the software package SIMCA-P for statistical modelling. *Mouse-models*: The experiments were performed using C57BL/6 wild-type and TLR4 deficient mice. A total of 44 metabolites were profiled in serum. *Bacterial infections:* 1 ml of the bacterial suspension prepared in saline (1 x 10^7 CFU/ml for *S. aureus* and *E. coli* and 1 x 10^6 CFU/ml for *P. aeruginosa*) were injected intraperitoneally. After 4 h and 24 h of infection, the distribution of the bacterial infection was monitored by bioluminescence and 300 µl of serum was collected by cardiac puncture and included in the NMR study. *Bacterial footprints*: Bacteria were cultured in LB medium and separated by filtration (0.2 µm) at an OD600 of 0.6. Culture media were analyzed directly by NMR to define the bacterial footprints.

Results: In our experiments we obtained significantly different ^{1}H NMR spectra for serum of *E. coli*, *S.aureus* and *P.aeruginosa* infected wild-type mice (Fig.1A). The corresponding profiles represent both metabolites activated by the innate defense system in mice and bacterial metabolites that are released into biological fluids. By combining the results of the in vivo study with footprints of culture experiments the following potential bacteria-specific biomarkers were identified: glutamate, proline, O-acetycarnitine for *S. aureus*, acetone, ethanol, and alanine for *E.coli* and isoleucine, leucine, ornithine, tryptophane and isobutyrate for *P.aeruginosa* infections. Serum of *E. coli* infected mice and of mice treated with LPS showed similarities in a wide range of metabolites and differences mainly occurred by the bacterial footprint. In TLR4 deficient mice the immune response upon LPS treatment was suppressed and only the metabolomic profile of the control group and mice with *E. coli* infection could be distinguished (Fig.1B). The corresponding metabolites are shown in the coefficient plot (Fig.2). Among the most significant metabolites were again the identified biomarkers of ethanol, alanine and acetone which are released as footprints into serum by *E. coli*.

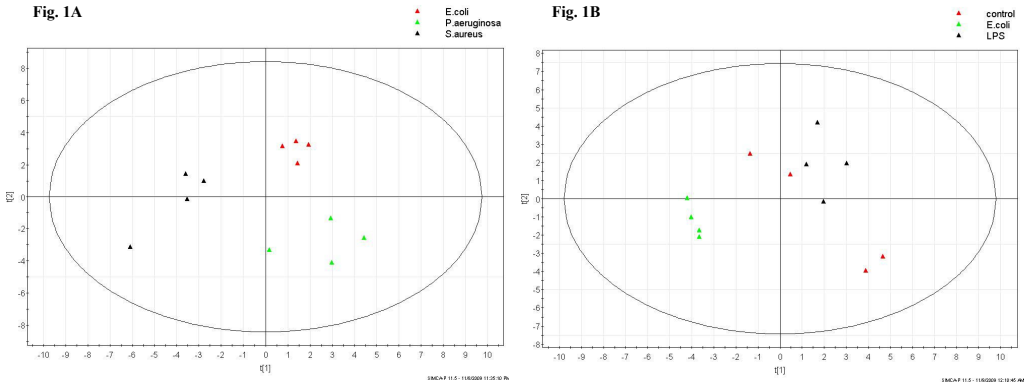
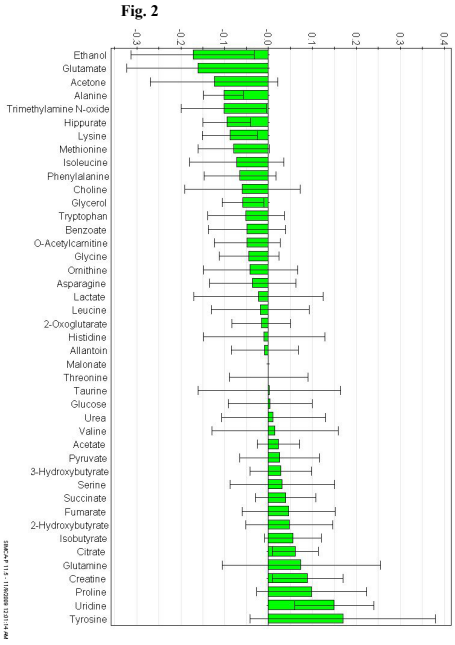


Fig.1: Partial least squares discriminant analysis (PLS-DA) scores plots, representing ^{1}H NMR spectral data from *E. coli*, *S. aureus* and *P. aeruginosa* infected C57BL/6 wild-type mice, showing the separation between samples along the type of infection (Fig.1A) and scores plot of the metabolite concentrations found in TLR4 deficient mice with LPS treatment and *E. coli* infection (Fig.1B). Two components encapsulate 82 % **(A)** and 90 % **(B)** of the interclass variation. **Fig.2**: The coefficient plot shows the relative contribution that each metabolite makes to the difference between the classes of the PLS-plot in Fig.1B.



Conclusion:

- The results show that the wide variety of compounds determined by the metabolomics approach helps to understand the pathological mechanism of bacterial infections in mammals.
- Both in C57BL/6 wild-type mice and TLR4 deficient mice, bacterial footprints correlate with the metabolites found in serum of infected mice and allowed for the identification of bacteria-specific biomarkers.

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