various intestinal and extraintestinal diseases. It is a known fact that the digestive tract may serve as a portal of entry into the bloodstream. Therefore, the presence of highly resistant *E. coli* strains in the gut presents a threat to the patients with predispositions such as chronic illnesses and poor immune status.
Methods: This study was undertaken with the aim to determine the resistance pattern among the *E. coli* strains isolated from the gut of chronically ill patients across wide clinical settings. The study was conducted over a period of 1 year from 1 January 2011 to 31 December 2011. Stool samples from patients admitted for more than 14 days in wards of all major clinical specialities were collected after proper counselling and informed consent. *E. coli* were identified on the basis of cultural characteristics and biochemical reactions. Strains isolated from pediatric patients were subjected to serotyping by the slide agglutination test with specific antisera (Denka Seiken Co., Ltd, Tokyo, Japan) to identify the enterovirulent strains. All *E. coli* strains were subjected to antimicrobial susceptibility testing and ESBL identification by the disk diffusion methods in accordance with the CLSI guidelines.
Results: Two hundred and fifty-four patients were included in the study with the following distribution: 71 from a pediatrics ward, 62 from a general medicine ward, 54 from a general surgery ward, 42 from a gynaecology ward, 16 from an orthopaedics ward and nine from an ENT ward. *E. coli* was isolated from 112 samples. Out of 34 *E. coli* strains isolated from paediatric patients, 14 were determined to be enterovirulent *E. coli* by serotyping. Antimicrobial susceptibility testing of all *E. coli* strains showed 100% resistance to nalidixic acid and a high degree of resistance to ampicillin (87.5%), doxycyline (83.0%), cotrimoxazole (75.9%), ciprofloxacin (73.2%) and third-generation cephalosporins (71.4%). ESBL production was detected in 71 strains (63.4%). However, no resistance was found for carbapenems and tigecycline.
Conclusion: A large population of chronically ill patients who were tested was found to be carrying highly resistant *E. coli* in their guts. These usually commensal strains may serve as a source of bloodstream infection especially in cases of immunosuppression. Whether these strains were acquired in the hospital or from the community needs to be studied further.

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5-Lipoxygenase contributes to PPARγ activation in macrophages in response to apoptotic cells

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Background: One hallmark contributing to immune suppression during the late phase of sepsis is macrophage polarization to an anti-inflammatory phenotype upon contact with apoptotic cells (AC). Taking the important role of the nuclear receptor PPARγ for this phenotype switch into consideration, it remains elusive how AC activate PPARγ in macrophages. Therefore, we were interested to characterize the underlying principle.
Methods: Apoptosis was induced by treatment of Jurkat T cells for 3 hours with 0.5 μg/ml staurosporine. Necrotic cells (NC) were prepared by heating cells for 20 minutes to 65°C. PPARγ activation was followed by stably transducing RAW264.7 macrophages with a vector encoding the red fluorescent protein mRuby after PPARγ binding to 4 × PPRE sites downstream of the reporter gene sequence. This readout was established by treatment with the PPARγ agonist rosiglitazone (1 μM) and AC (5:1). Twenty-four hours after stimulation, mRuby expression was analysed by fluorescence microscopy. Lipid rafts of AC, NC, as well as living cells (LC) were enriched by sucrose gradient centrifugation. Fractions were analysed for lipid raft-associated marker proteins. Lipid rafts were incubated with transduced RAW264.7 macrophages as described above. 5-Lipoxygenase (5-LO) involvement was verified by pharmacological inhibition (MK-866, 1 μM) and overexpression.
Results: Assuming that the molecule responsible for PPARγ activation in macrophages is localized in the cell membrane of AC, most probably associated to lipid rafts, we isolated lipid rafts from AC, NC and LC. Mass spectrometric analysis of lipid rafts of AC showed the expression of 5-LO, whereas lipid rafts of LC did not. Moreover, incubating macrophages with lipid rafts of AC induced mRuby expression. In contrast, lipid rafts of NC and LC did not. To verify the involvement of 5-LO in activating PPARγ in macrophages, Jurkat T cells were incubated for 30 minutes with the 5-LO inhibitor MK-866 (1 μM) before apoptosis induction. In line with our hypothesis, these AC did not induce mRuby expression. Finally, although living Jurkat T cells overexpressing 5-LO did not activate PPARγ in macrophages, mRuby expression was significantly increased when AC were generated from 5-LO overexpressing compared with wild-type Jurkat cells.
Conclusion: Our results suggest that induction of apoptosis activates 5-LO, localizing to lipid rafts, necessary for PPARγ activation in macrophages. Therefore, it will be challenging to determine whether 5-LO activity in AC, generated from other cell types, correlates with PPARγ activation, contributing to an immune-suppressed phenotype in macrophages.

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Natural killer cell status and tolerance in mouse and human bacterial sepsis

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Background: As sensors of infection, innate immune cells are able to recognize pathogen-associated molecular patterns by receptors such as Toll-like receptors (TLRs). Natural killer (NK) cells contribute to inflammatory processes by producing proinflammatory cytokines such as IFNγ and GM-CSF [1]. Our aim was to characterize the immune status of NK cells in a murine model of sepsis and in patients with systemic inflammatory response syndrome (SIRS) and sepsis.
Methods: Cecal puncture (CP) was employed as a murine model of polymicrobial sepsis. TLR expression in murine and human NK cells was studied by flow cytometry. *Ex vivo* IFNγ production was analyzed either by ELISA or by flow cytometry.
Results: In mice, the expression of TLR2 and TLR4 in spleen NK cells is mainly intracellular, similarly to TLR9. *In vitro* cell responsiveness of purified NK cells to TLR2, TLR4 or TLR9 agonists, in synergy with accessory cytokines (IL-2, IL-15 and IL-18), allowed a significant production of IFNγ and GM-CSF. In contrast, NK cells, purified from spleen of mice with sepsis, showed a dramatic reduction of their capacity to produce cytokines in response to TLR agonists. Depletion of regulatory T cells (Tregs) before CP led to a complete reversion of NK cell tolerance to TLR agonists. IL-10 and TGF-β1 are two main inhibitory cytokines produced by Tregs. We showed *in vivo*, using IL-10 knockout mice and by inhibiting TGF-βR signaling, that the tolerization mechanism of NK cells was mostly mediated by TGF-β [2]. In humans, the expression of TLR2, TLR4 and TLR9 in peripheral blood NK cells (both $CD3^-CD56^{high}$ and $CD3^-CD56^{dim}$ subsets) was mainly intracellular. The *ex vivo* responsiveness of the blood NK cells to their agonists in synergy with accessory cytokines (IL-15 and IL-18), allowed a significant secretion of IFNγ. Similar to the murine model of sepsis, in SIRS and sepsis patients the secretion of IFNγ by NK cells was significantly decreased.
Conclusion: NK cells express TLR2 and TLR4 intracellularly, as already reported for other cell types (epithelial, endothelial, and dendritic cells). Furthermore, NK cells undergo tolerance to TLR agonists during SIRS or sepsis, as already described for monocytes in these clinical settings.
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Raman spectroscopic investigation of the interaction of *Enterococcus faecalis* and vancomycin: towards a culture-independent antibiotic susceptibility test

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