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 specialties 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 the four hours 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%), doxycycline (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.

P84 Natural killer cell status and tolerance in mouse and human bacterial sepsis
F Souza-Fonseca-Guimaraes1, 7, M Parlato1, F Philippart2, B Missert2, J M Cavallion1, M Adib-Conquy1, G Captain Study Group1, D Steinhilber2
1 Institut Pasteur, Paris, France; 2 Groupe hospitalier Paris Saint Joseph, Paris, France
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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 IFN-γ 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 IFN-γ 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-β signaling, that the tolerization mechanism of NK cells was mostly mediated by TGF-β1 [2]. In humans, the expression of TLR2, TLR4 and TLR9 in peripheral blood NK cells (both CD3+ CD56dim and CD3+ CD56dim 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 IFN-γ. Similar to the murine model of sepsis, in SIRS and sepsis patients the secretion of IFN-γ 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.

References

P85 Raman spectroscopic investigation of the interaction of Enterococcus faecalis and vancomycin: towards a culture-independent antibiotic susceptibility test
U Neugebauer2, 1, C Assmann1, U Schroder1, A Ramoji1, U Glassel1, C Beleites1, W Pfitzer1, J Popp1, M Bauer1
1 Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany; 2 Institute of Photonic Technology, Jena, Germany; 3 Institute of Medical Microbiology, Jena University Hospital, Jena, Germany; 4 Institute of Photonic Technology, Institute of Physical Chemistry and Abbe Center of Photonics, Jena, Germany
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Background: The bacterial cell wall plays an essential role in the expression of several virulence factors. Resistance to antibiotics is an emerging problem and plays a substantial role in the increasing mortality rate. The cell wall of Gram-positive and Gram-negative bacteria is composed of peptidoglycan, which is susceptible to antibiotics. The interactions of antibiotics with the cell wall of antibiotics-susceptible and -resistant strains can be monitored by Raman spectroscopy, which has been demonstrated for Neisseria meningitidis, Staphylococcus aureus, and Enterococcus faecalis. Non-labeled Raman spectroscopy can be used in combination with a culture-independent method for antibiotic susceptibility testing.

Methods: Raman spectroscopy was used to investigate the effects of vancomycin on a sensitive E. faecalis strain and a selected resistant strain. The cell wall composition of both strains was compared following their exposure to vancomycin.

Results: The Raman spectroscopy measurements confirmed the known composition of the cell wall of E. faecalis. The peaks at 1250 cm⁻¹ and 1650 cm⁻¹ were assigned to β1,3-glucans and muramic acid, respectively. The peak at 2900 cm⁻¹ was due to lipid A. The susceptibility of E. faecalis to vancomycin was confirmed by the reduced intensity of the peak at 1650 cm⁻¹, which represents the muramic acid content. The interaction of vancomycin with the cell wall of E. faecalis resulted in a decrease in peak intensity at 1650 cm⁻¹, indicating the inhibition of cell wall synthesis. In contrast, the resistant strain showed no change in the peak intensity at 1650 cm⁻¹ upon exposure to vancomycin, indicating the absence of cell wall synthesis inhibition.

Conclusion: Raman spectroscopy can be used as a culture-independent method for antibiotic susceptibility testing. This method allows the rapid and non-invasive detection of antibiotic resistance in bacteria. The results obtained in this study suggest that Raman spectroscopy can be a valuable tool for the development of new antibiotics and the monitoring of their efficacy in combination with conventional susceptibility tests.