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Vendor effects on murine gut microbiota influence experimental abdominal sepsis

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ABSTRACT

Background: Experimental animal models are indispensable components of preclinical sepsis research. Reproducible results highly rely on defined and invariant baseline conditions. Our hypothesis was that the murine gut microbiota varies among different distributors of laboratory animals and that these variations influence the phenotype of abdominal sepsis derived from a bacterial inoculum model (intraperitoneal stool injection).
Materials and methods: Male C57BL/6 mice (8-wk old) purchased from Charles River (CR), Janvier (J), and Harlan (H) were sacrificed, and the bacterial composition of feces was analyzed using CHROMagar orientation medium. Stool was injected intraperitoneally into CR mice, followed by clinical observation and gene expression analysis. Experiments were repeated 16 mo later under the same conditions.
Results: Stool analysis revealed profound intervendor differences in bacterial composition, mainly regarding *Staphylococcus aureus* and *Bacillus licheniformis*. Mice challenged with CR as well as H feces developed significantly higher severity of disease and died within the observation period, whereas stool from J mice did not induce any of these symptoms. Real-time polymerase chain reaction revealed corresponding results with significant upregulation of proinflammatory cytokines and vascular leakage-related mediators in CR and H injected animals. Sixteen months later, the bacterial fecal composition had significantly shifted. The differences in clinical phenotype of sepsis after intraperitoneal stool injection had vanished.
Conclusions: We are the first to demonstrate vendor and time effects on the murine fecal microbiota influencing sepsis models of intraabdominal stool contamination. The

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intestinal microbiota must be defined and standardized when designing and interpreting past and future studies using murine abdominal sepsis models.

Introduction

Experimental animal models are indispensable components of preclinical sepsis research. They help to validate hypotheses generated *in vitro*, translating findings into a more complex organism. A wide range of various animal models to study bacterial sepsis have been developed over the last decades, predominantly using mice.[1] Apart from the simple injection of pathogenic ligands such as lipopolysaccharide (LPS, "endotoxin"), flagellin, or CpG oligodeoxynucleotides, the transfer of bacteria into the lung or the abdominal cavity is widely used to induce the clinical and molecular phenotype of gram-positive and -negative sepsis. Popular models to generate sepsis from an abdominal focus are, among others, the intraperitoneal stool injection (IPSI), the cecal ligation and puncture (CLP), and the colon ascendens stent peritonitis (CASP) model.[2] Although CLP and CASP are models of endogenous fecal contamination, being based on induced stool leakage into the peritoneal cavity, for the IPSI model, feces previously harvested from another living being are injected through the abdominal wall. Regardless of the model used, reproducible results rely on defined and invariant baseline conditions, that is to say primarily the bacterial composition of the feces being used to create an intraabdominal focus. In human sepsis, it was demonstrated that the bacterial spectrum isolated from the abdomen is associated with the patients' outcome.[3] Moreover, it is known that the indigenous intestinal flora itself influences the individual's susceptibility to infection.[4] On the other hand, this intestinal flora may be subject to change by varying environmental conditions.[5] Our hypothesis was that the murine gut microbial flora varies among different distributors of laboratory animals and that these variations influence the phenotype of experimental abdominal sepsis. We therefore analyzed the intestinal bacterial composition of one same inbred strain (C57BL/6 mice) from different vendors and examined the clinical as well as molecular manifestation of sepsis derived from corresponding IPSI. Our results will contribute to the definition of parameters for standardization and help designing and interpreting past and future studies using murine models of experimental abdominal bacterial sepsis.

Materials and methods

Animals

All experiments were performed on C57BL/6 mice (*n* substrain) at an age of about 8 wk. Only animals of male sex were used to control for hormonal variations that may have obfuscated the results, thus ensuring the necessary validity of the study. Stool donor mice were purchased from Charles River (CR; Sulzfeld, Germany), Janvier (J; LeGenest-Saint-Isle, France), and Harlan (H (now Envigo Research Models and Services); Indianapolis, Indiana, USA) and were sacrificed immediately after purchase without prior housing in the local animal facility. Recipient animals were purchased from CR and J and were housed for 14 d in individually ventilated, pathogen-free cages with access to water and standard rodent chow (provided from SSNIFF GmbH, Soest, Germany) ad libitum before challenge. The animal protocol of this study was approved by the local committee for animal care (LANUV, Recklinghausen, Germany; protocol no. 84-02.04.2013.A071) and was in accordance with the National Institutes of Health guidelines for the use of live animals (NIH publication No. 85-23, revised 1996).

Isolation of stool from donor mice

Donor mice were sacrificed by cervical dislocation immediately after purchase without prior housing in the local animal facility. After median laparotomy, stool from the cecum and colon was collected under sterile conditions. One animal yielded approximately 0.1-0.2 g of feces. Stool from individual animals was analyzed for bacterial composition, whereas for the challenge experiments, the stool from 20 mice per vendor was pooled.

Initial experiments were performed in 2012 (October) and were subsequently repeated 16 mo later (February 2014).

Analysis of bacterial composition of stool

For initial characterization of the microbial composition of murine feces, freshly isolated stool from donor mice was inoculated on CHROMagar orientation medium plates (CHROMagar, Paris, France) and incubated under controlled aerobic conditions at 37°C for 48 h at the Institute for Medical Microbiology, Immunology and Parasitology (IMMIP) of the University Hospital Bonn according to the local standard. This medium was designed for rapid discrimination of different bacterial pathogens by the color of the colonies formed on the agar. The differentiation was performed according to the manufacturer's description. Since a blue appearance should indicate both bacteria from the *Klebsiella (*KES) group (*Klebsiella*, *Enterobacter*, *Citrobacter,* and *Serratia* spp.) as well as *enterococci*, these groups were summarized and termed "KES-E". Random samples of colonies of all colors were furthermore analyzed using Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass Spectrometry to verify the correct identification of the corresponding bacterial species.

Feces from 20 donor animals of each vendor were analyzed, and the number of mice showing positive results for the individual bacterial species was used to calculate the relative abundance in percentage of total readouts.

Challenge experiments: intraperitoneal stool injection

For intraperitoneal application, freshly isolated stool samples from 20 mice from each of the three different distributors were pooled within each group and diluted 1:3 using sterile 0.9% sodium chloride solution. Large particles were removed by filtration, yielding a suspension that easily passes through a