aGvHD

Lactose-driven Enterococcus expansion linked to unfavorable prognosis in GvHD

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Patients undergoing allogenic hematopoietic stem cell transplant (allo-HSCT) have been shown to display a reduced diversity and marked shift in their intestinal microbiota. In particular, commensal *Enterococcus* species are suggested to dominate the fecal microbiota of patients following allo-HSCT, while levels of *Clostridium* are speculated to deplete.[1] The butyrate-forming functionality of commensal clostridia provides protection against graft-*versus*-host disease (GvHD).[2] Disruption of normal gut microbiota, caused by broad-spectrum antibiotics, has been associated with increased incidence of lethal GvHD and poor overall survival (OS) in humans and mice.[3] Pre-clinical mouse studies have uncovered that *Enterococcus* species are involved in the initiation of antigen-presenting cell (APC) and $CD4^+/ROR\gamma^+$ T-cell infiltration that results in the establishment of colitis.[4]

A study by Stein-Thoeringer, Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY, US, and colleagues, investigated the role of enterococci in the establishment of acute GvHD (aGvHD) in both allo-HSCT recipients and pre-clinical allo-HSCT mouse models. The study also explores the theory that *Enterococcus* vitality is reliant on lactose metabolism and looks at the effect of dietary lactose and lactose-malabsorption genotypes on the severity of GvHD in humans and mice.[5]

MULTICENTER PATIENT STUDY

Study design

- Patients across four transplant centers underwent allo-HSCT following ablative, reduced intensity or nonmyeloablative conditioning (**Table 1**)
- Patients were divided into two main observation cohorts:
  1. MSKCC cohort
  2. Multicenter validation cohort: Duke University (US), Hokkaido University (Japan) and University Hospital Regensburg (Germany)
- Patient fecal microbiota was profiled using 16S rRNA gene sequencing over the course of allo-HSCT (day -30 to +24 relative to HSCT; 7-day sliding windows)
- *Enterococcus* domination was defined as relative genus abundance of ≥30% in any fecal sample
- Pre- and post-transplant fecal samples were collected from patients receiving allo-HSCT for acute myeloid leukemia (AML) in the MSKCC cohort to investigate the microbial metabolic pathways that characterize domination
- Identification of patients carrying a lactose malabsorption genotype was carried out using the single nucleotide polymorphism (SNP), rs4988235(-13910*T) in 602 patients from the MSKCC cohort with available pretransplant

germline DNA samples

Results

- Patient characteristics
  - In total 1,325 adult allo-HSCT recipients were recruited across the four centers:
    - 1,101 patients in MSKCC cohort
    - 224 patients in the multicenter validation cohort

Table 1. Clinical characteristics of the patient cohort

Overall Cohort	**N=1,325**
Institution (%)	
MSKCC	1,101 (83.1)
Regensburg	79 (6.0)
Duke	79 (6.0)
Hokkaido	66 (5.0)
Age at HSCT, year (mean (sd))	52.9 (12.8)
Sex (male, %)	801 (60.5)
Disease (%) AML	485 (36.6)
MDS/MPN	244 (18.4)

NHL	223 (16.8)
ALL	124 (9.4)
Myeloma	113 (8.5)
CLL	33 (2.5)
CML	29 (2.2)
Hodgkins	30 (2.3)
AA	9 (0.7)
Other	35 (2.6)
Graft type (%)	
BM/PBSC unmodified	660 (49.8)
Cord	207 (15.6)
PBSC T-cell depleted	458 (34.6)
Conditioning intensity (%)	
Ablative	744 (56.2)
Reduced intensity	466 (35.2)
Nonmyeloablative	115 (8.7)

AA, aplastic anemia; ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; BM, bone marrow; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HSCT, hematopoietic stem cell transplantation; MDS/MPN, myelodysplastic syndromes/myeloproliferative neoplasms; MSKCC, Memorial Sloan Kettering Cancer Centre; NLH, Non-Hodgkin Lymphomas; PBSC, peripheral blood stem cells; sd, standard deviation.

- Microbiota domination
  - *Enterococcus* domination was observed in 65% of patients after allo-HSCT across centers
  - *E. faecium* was the dominant enterococcal species across both cohorts, with 40.1% and 46.0% of patients in the MSKCC and multicenter-validation cohorts respectively, reaching domination between days -20 and +80 of allo-HSCT
  - *Enterococcus* fecal domination in the early allo-HSCT period (day 0 to +21 post allo-HSCT) was associated with significantly reduced OS in both the MSKCC (HR 1.97; p< 0.001) and combined multicenter-validation (HR 1.95; p= 0.03) cohorts
  - *Enterococcus* fecal domination in the early allo-HSCT period was associated with increased GvHD-related mortality in both the MSKCC (HR 2.04; p= 0.01) and combined multicenter-validation (HR 5.8; p= 0.002) cohorts
- Lactose and *Enterococcus:*
  - Metabolic pathways involved in lactose and galactose degradation were enriched in *E. faecium*-dominated, post-transplant microbiota but were not common in pre-transplant samples
  - Enterococcal expansion following allo-HSCT was associated with a reduction of *Clostridium* and fecal butyrate
  - Enterococcal domination was significantly prolonged in patients with lactose malabsorption following termination of broad-spectrum antibiotics when compared to those patients that had lactose absorber genotypes

PRE-CLINICAL MOUSE STUDY

Study Design

- Three mouse models of allo-HSCT were utilized to investigate GvHD related mortality:
  1. Major histocompatibility complex (MHC)-matched, minor-antigen-mismatched allo-HSCT (C57BL/6-to-129S1/Sv transplant [C57BL/6→129S1/Sv])
  2. MHC-disparate model after irradiation conditioning (C57BL/6→ BALB/c)
  3. MHC-matched, minor antigen-mismatched after busulfan and cyclophosphamide conditioning (LP/J→ C57BL/6)
- Mice received bone marrow (BM) or T cell-replete bone marrow (BM+T [2×10^6 T cells])
- 16S rRNA gene sequencing was carried out on fecal samples
- Microbiota domination and GvHD:
  - LP/J→ C57BL/6 mice were colonized with a community of six bacterial strains (*Akkermansia muciniphila, Lactobacillus johnsonii, Blautia producta, Bacteroides sartorii, Clostridium bolteae, and Parabacteroides diastonis*) on day -21 of allo-HSCT (LP/J→ gnotobiotic C57BL/6)
    - One group of mice then underwent *E. faecalis OG1RF* cocolonization on day -21 of allo-HSCT
    - One group of mice then underwent *E. faecalis OG1RF* cocolonization post-transplant

- Lactose, *Enterococcus* and GvHD:
  - Lactose-free chow was fed to C57BL/6→BALB/c and LP/J→ C57BL/6 mice
  - Mice were subjected to chemotherapy conditioning at days-7 to -3 relative of HSCT
  - Flow cytometric analysis of donor T-cells was carried out on day +14 of allo-HSCT

Results

- Microbiota domination and GvHD:
  - Across the gnotobiotic mouse models, *Enterococcus* dominated the gut microbiota at day +8 of HSCT of all mice that developed aGvHD
  - *E. faecalis* expansion was independent of antibiotic administration and dependent on GvHD development, as it was not seen in control mice receiving T cell–depleted allografts and therefore did not develop GvHD
  - Mice receiving BM+T allo-HSCT elicited significantly reduced OS in mouse models 1 ($p= 0.013$), 2 ($p< 0.001$) and 3 ($p< 0.01$), compared to those receiving BM allo-HSCT
  - *E. faecalis* colonization resulted in significantly elevated interferon-γ serum concentrations
  - *E. faecalis* colonization resulted in increased numbers of donor T-cells, activated $CD4^+$ T-cells and $CD4^+RORg^+$ T helper 17 (T_H17) cells in colon lamina propria
  - Post-transplant administration of *E. faecalis OG1RF* significantly reduced OS ($p< 0.001$) and aggravated GvHD in mice receiving BM+T allo-HSCT
- Lactose, *Enterococcus* and GvHD:
  - Metabolic pathways involved in lactose and galactose degradation were enriched in *E. faecalis*–dominated, post-transplant microbiota of mice with GvHD
  - Enterococcal expansion was associated with a reduction in *Clostridium* and fecal butyrate in mice with GvHD
  - A lactose-free diet significantly reduced the abundance of post-transplant *Enterococcus* and incidence of experimental GvHD
  - A lactose-free diet resulted in a reduction of activated and proliferating $CD4^+$ T-cells and $CD4^+Tbet^+$ (T_H1) T-cells
  - A lactose-free diet significantly increased post-transplant survival (%) in mice that had undergone allo-HSCT, both with ($p<0.01$) or without ($p<0.05$) prior chemotherapeutic conditioning

Conclusions

- Fecal *Enterococcus* species domination following allo-HSCT is a risk factor for aGvHD development and increased GvHD-associated mortalities
- Lactose fuels the expansion of enterococci and could be targeted to prevent intestinal and systemic T-cell activation with subsequent development of diseases such as GvHD
- Maintenance of enterococcal domination following administration of broad-spectrum antibiotics may be reduced by restricting the intake of lactose
- Observations from the study provide rationale for a lactose-free diet, to improve patient outcome following allo-HSCT

References

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