Integrated meta-omic analyses of the gastrointestinal tract microbiome in patients undergoing allogeneic hematopoietic stem cell transplantation

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In patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), treatment-induced changes to the gastrointestinal tract (GIT) microbiome have been linked to adverse outcomes, most notably graft-versus-host disease (GvHD). However, it is presently unknown whether this relationship is causal or consequential. Here, we performed an integrated meta-omic analysis to probe deeper into the GIT microbiome changes during allo-HSCT and its accompanying treatments. We used 16S and 18S rRNA gene amplicon sequencing to resolve archaea, bacteria, and eukaryotes within the GIT microbiomes of 16 patients undergoing allo-HSCT for the treatment of hematologic malignancies. These results revealed a major shift in the GIT microbiome after allo-HSCT including a marked reduction in bacterial diversity, accompanied by only limited changes in eukaryotes and archaea. An integrated analysis of metagenomic and metatranscriptomic data was performed on samples collected from a patient before and after allo-HSCT for acute myeloid leukemia. This patient developed severe GvHD, leading to death 9 months after allo-HSCT. In addition to drastically decreased bacterial diversity, the post-treatment microbiome showed a higher overall number and higher expression levels of antibiotic resistance genes (ARGs). One specific *Escherichia coli* strain causing a paravertebral abscess was linked to GIT dysbiosis, suggesting loss of intestinal barrier integrity. The apparent selection for bacteria expressing ARGs suggests that prophylactic antibiotic administration may adversely affect the overall treatment outcome. We therefore assert that such analyses including information about the selection of pathogenic bacteria expressing ARGs may assist clinicians in "personalizing" regimens for individual patients to improve overall outcomes. (Translational Research 2017;186:79–94)

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Abbreviations: aGvHD = acute graft-versus-host disease; allo-HSCT = allogeneic hematopoietic stem cell transplantation; ARG = antibiotic resistance gene; ATG = antithymocyte globulin; bp = base pair; cDNA = complementary DNA; RHM = reference healthy microbiome; contig = contiguous sequence; GIT = gastrointestinal tract; GvHD = graft-versus-host disease; IMP = Integrated Meta-omic Pipeline; MG = metagenomic; MT = metatranscriptomic; NCBI = National Center for Biotechnology Information; nt = nucleotide; OTU = operational taxonomic unit; PAMP = pathogen-associated molecular pattern; rRNA = ribosomal RNA; SCFA = short-chain fatty acid; SNV = single nucleotide variant; TP = time point

AT A GLANCE COMMENTARY

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Background

Allogeneic hematopoietic stem cell transplantation is a therapy for many hematological malignancies. However, its effects on the gastrointestinal tract microbiome remain poorly understood.

Translational Significance

- Allogeneic hematopoietic stem cell transplantation and its associated drug treatments have detrimental effects on the gastrointestinal tract microbiome.
- Antibiotic treatment of these patients gives rise to overgrowth of pathogenic microbes, possessing and expressing antibiotic-resistant genes.
- Loss of epithelial barrier function likely contributes to bacterial and fungal invasions and activation of inflammatory responses.
- Enrichment in potentially pathogenic strains through accompanying antibiotic treatment may further contribute to worsening overall treatment outcome through systemic infection.
- Pathogenic microbes might provide antigens to antigen-presenting cells, activating various immune effectors that associate with acute graft-versus-host disease.

INTRODUCTION

Humans live in a close ("symbiotic") relationship with an inherent "microbiome", comprised of bacteria, archaea, and unicellular eukaryotes. The most densely populated human body habitat is the gastrointestinal tract (GIT).[1] The GIT microbiome plays a myriad of roles vital to human physiology, including digestion of food, synthesis of vitamins, production of short-chain fatty acids, and the prevention of colonization by pathogens through exclusion.[2] In a healthy human GIT, microorganism homeostasis is tightly regulated by the host's immune system.[3-5] However, various perturbations, such as antibiotic attenuation of sensitive bacteria, may disrupt this balanced state, leading to a state typically referred to as "dysbiosis",[3,6] in which pathogenic microbes can overgrow the community.[6] Furthermore, reduced intestinal barrier function can facilitate translocation of microorganisms and microbial products from the GIT lumen to mesenteric lymph nodes and/or the bloodstream,[7] putting the host at risk for local infections and sepsis.[6,8]

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents an effective treatment for several hematological malignancies. Transplantation is preceded by a conditioning regimen, consisting of either total body immune ablative irradiation or high doses of chemotherapy, to facilitate engraftment of transplanted stem cells. Moreover, allo-HSCT is known to greatly impact the stability and integrity of the GIT microbiome, resulting in substantially reduced bacterial diversity and the emergence of dominance by (often drug-resistant) single bacterial taxa.[9]

The conditioning treatment for allo-HSCT may also lead to mucositis of the GIT, culminating in the formation of ulcers, dysphagia, and diarrhea.[10] One complication of allo-HSCT is acute graft-versus-host disease (aGvHD),[11] a systemic, inflammatory disease that is provoked by a complex allogeneic immune response, primarily affecting the skin, liver, and GIT.[12] In addition, the GIT microbiome has been implicated in the development and/or exaggeration of aGvHD. Specifically, the damaged GIT epithelial barrier in allo-HSCT patients allows translocation of microorganisms or pathogen-associated molecular patterns (PAMPs).[13] PAMPs can then activate antigen-presenting cells, leading to alloactivation and proliferation of donor T cells that trigger aGvHD.[13]

Supportive care of patients receiving allo-HSCT includes prophylactic broad-spectrum antibiotic treatment,[14] an intervention that also selects for potential pathogens carrying antibiotic resistance genes (ARGs),[15] within the GIT microbiome. Such antibiotics also drive horizontal transfer of ARGs among commensal bacteria, often including numerous opportunistic pathogens.[16] Antibiotic treatment has ambiguous effects on treatment outcome. On the one hand, a low bacterial diversity at engraftment, possibly caused by chemotherapy, total body irradiation, and/or broad-spectrum antibiotics, has been linked to