

Stem cell transplantation - Section 17

## Microbiota and Graft-versus Host disease: a double-edged sword

Ernst Holler, Daniela Weber

Department of Internal Medicine 3, University Medical Center, Regensburg, Germany

### Take home messages

- HSCT patients have a high likelihood of severe dysbiosis early after transplantation which is mediated by broad spectrum antibiotics and GvHD itself while real decontamination is rarely achieved.
- Commensal microbiota play a major role in maintaining immunological homeostasis in the gut, but also systemically.
- Microbiota modulation offers a new option for treatment or even prophylaxis of GvHD.

### Introduction

Acute and chronic Graft-versus Host disease (GvHD) are still the major causes of morbidity and mortality following allogeneic stem cell transplantation (HSCT). Although donor cell activation by recipient antigens is in the center of its pathophysiology, the preferential manifestation of GvHD at epithelial surfaces already points to an important modulator of GvHD-related inflammation—the microbiota consisting of both, commensal bacteria and pathogens. The contribution of intestinal bacteria to inflammation in GvHD was well known for many years since van Békúm’s observation on reduction of GvHD in germfree mice<sup>1</sup> and identification of bacterial lipopolysaccharide as an important costimulatory molecule in GvHD.<sup>2</sup> However, these findings were observed in an era where analysis of microbiota—host interaction was restricted to culturable bacteria. With the introduction of 16s rRNA molecular sequencing of bacteria, it became clear that the large group of anaerobic commensal bacteria had not been integrated in this interaction before. Here we summarize how current approaches of microbiota research changed our view on pathophysiology of HSCT-related complications and discuss the potentials and risks of microbiota-based interventions in the setting of GvHD.

### Current state of the art

The 16s molecular sequencing of stool microbiota in HSCT patients was first reported by the Memorial Sloan Kettering Cancer Center<sup>3</sup> and later on by our group<sup>4</sup>: In both studies, loss of bacterial diversity and commensal bacteria was observed in the first 2 weeks after transplantation, which was associated with an increased risk of GvHD and long-lasting transplant mortality. These studies also showed that antibiotic prophylaxis which was for a long time considered as standard of care and helpful not only in prophylaxis of infections but also of GvHD, rarely achieves complete decontamination but results in massive loss of diversity (dysbiosis) with abundance of single pathogenic bacteria. Since these first reports numerous studies confirmed these associations (review in Andermann et al.<sup>5</sup>) and defined antibiotics used for prophylaxis and treatment of infections as a major cause of dysbiosis. A puzzling question is how microbiota changes in the first 2 weeks can exert such a long-lasting impact on outcome: A possible explanation is that dysbiosis occurs at the time of rebuilding of the immune system by donor cells—thus HSCT repeats the immunological conditions occurring early in neonatal life where also a high vulnerability by dysbiosis has been shown.<sup>6</sup>

A major challenge was to define mechanisms explaining this impact of dysbiosis on alloreaction and long-term immunodeficiency: As in other areas of microbiota-associated diseases, metabolites produced by commensal bacteria are considered to mediate beneficial effects: short-chain fatty acids (SCFA) released after degradation of dietary fibers by commensal bacteria have been reported to be highly protective, and in an experimental study, P Reddy’s group nicely showed that GvHD protection by a 17-strain cocktail of commensal clostridia could be explained by the SCFA butyrate produced by these bacteria.<sup>7</sup> Bacterial tryptophan metabolites like indoles are other important immunoregulatory metabolites, which induce a tolerogenic dendritic cell type in vitro. Recently, Swimm et al used a specific indole derivative (indolecarbaldehyde) in mice and reported strong protection from GvHD-related pathology and strongly improved survival in mice receiving indolecarbal-

Funding/support: None.

Disclosure: E Holler is consulting MaatPharma, Lyon, France.

Copyright © 2019 the Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the European Hematology Association. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

HemaSphere (2019) 3:S2

Received: 29 January 2019 / Accepted: 8 March 2019

**Citation:** Holler E, Weber D. Microbiota and Graft-versus Host Disease: A Double-edged Sword. *HemaSphere*, 2019;3:S2. <http://dx.doi.org/10.1097/HS9.000000000000204>.

dehydrate prophylactically without interference with GvL effects.<sup>8</sup> As there is strong interaction of microbiota and microbiota-derived metabolites and intestinal regulatory T cells, this axis seems to be 1 predominant mechanism in regulation of GvHD (Fig. 1).

If GvHD is modulated by the microbiota, an important question is whether GvL is equally affected. Whereas experimental studies clearly see a differential effect of a diverse microbiome on GvHD and GvL, there is only 1 clinical study directly addressing this question: Indeed, presence of specific commensals such as *Eubacterium limosum* protects against relapse and thus supports the general concept that intestinal microbiota also regulate antitumor immunosurveillance.<sup>9</sup>

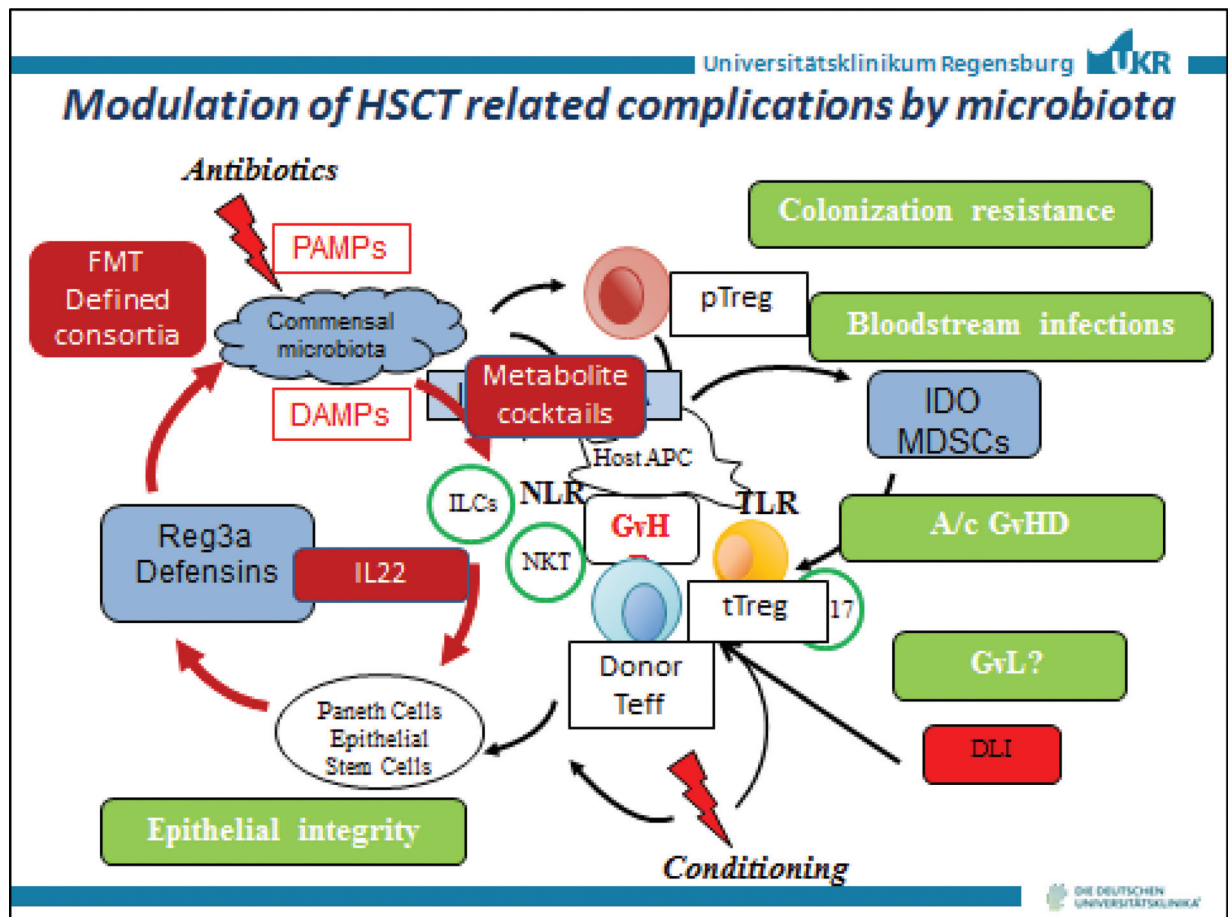
Currently, the most direct approach to restore commensal bacteria and microbiota diversity is fecal microbiota transfer (FMT) as it has become standard of care in severe relapsing C diff infections. However, there is significant concern that this may transfer potential infectious organisms to the highly vulnerable HSCT patients. On the other hand, there is now substantial evidence that restoration of a diverse microbiome protects against translocation of pathogenic bacteria and suppresses or even eliminates multiresistant bugs by restoration of colonization resistance, both events occurring frequently in advanced GvHD patients.<sup>10</sup> More recently, beneficial effects of diverse microbiota even on respiratory infections have been

shown, as abundance of butyrate producing commensals was protective against progression of respiratory viral infections in HSCT patients.<sup>11</sup>

In addition, first reports on either prophylactic or therapeutic application of FMTs in HSCT pts did not observe severe infectious complications after FMT. Both autologous and allogeneic third-party donor FMT have been successfully used for prophylactic restoration of diversity.<sup>12,13</sup> In addition, several pilot studies reported treatment of steroid refractory gastrointestinal GvHD by allogeneic FMT: Overall, up to 70% of pts responded with complete or at least partial resolution of GvHD symptoms<sup>14</sup> supporting the concept that intact intestinal immunoregulation needs a diverse microbiome on one hand and that current immunosuppressive strategies may eliminate alloreactive T cells but at the same time frequently prevent immune reconstitution as well.

**Future perspectives**

Although first results of FMT trials indicate efficacy, clear clinical trials on FMT with defined entry and outcome criteria are missing. Furthermore, it is currently unknown whether crude FMT from healthy donors just acts by yet unknown specific mechanisms delivered by defined bacterial strains or works via metabolic effects provided by a healthy microbiota. The interplay of the



**Figure 1. Modulation of HSCT related complications by microbiota.** FMT=fecal microbiota transfer, ILC=innate lymphoid cells, MDSCs= myeloid derived suppressor cells, NLR and TLR=Nod-like and Toll-like receptors, p/n T=reg induced and natural regulatory T cells, PAMPs and DAMPs=pathogen and damage associated molecular patterns.

donor versus recipient T cell repertoire against specific bacteria, as it has been recently shown in inflammatory bowel disease,<sup>15</sup> has even not been addressed in the setting of HSCT and the same is true for the specific IgA response which plays a major role in regulating microbiota but is severely suppressed in HSCT patients. Chronic GvHD manifestations occur most frequently at sites with specific microbiota/epithelial interactions (mouth, eye, skin, lung, urogenital GvHD) but these interactions need further evaluation. Finally, it is a huge clinical challenge to balance the need for anti-infectious, mostly antibiotic prophylaxis and treatment in immunosuppressed HSCT patients versus the detrimental effects of broad spectrum antibiotics on microbiota. More intelligent approaches like protecting the intestinal microbiome by antibacterial peptides or the recently reported approach to neutralize antibiotics in the gut using oral enzymes like beta-lactamases<sup>16</sup> may help to balance the needs in the future.

## References

1. van Bekkum DW, Roodenburg J, Heidt PJ, et al. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. *J Natl Cancer Inst.* 1974;52:401–404.
  2. Ferrara JL, Levine JE, Reddy P, et al. Graft-versus-host disease. *Lancet.* 2009;373:1550–1561.
  3. Jenq RR, Ubeda C, Taur Y, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med.* 2012;209:903–911.
  4. Holler E, Butzhammer P, Schmid K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. *Biol Blood Marrow Transplant.* 2014; 20:640–645.
  - \*5. Andermann TM, Peled JU, Ho C, et al. The microbiome and hematopoietic cell transplantation: past, present, and future. *Biol Blood Marrow Transplant.* 2018;24:1322–1340.
- This comprehensive review summarizes expert opinion on the status and needs of microbiota research in the setting of HSCT.**
6. Torow N, Hornef MW. The neonatal window of opportunity: setting the stage for life-long host-microbial interaction and immune homeostasis. *J Immunol.* 2017;198:557–563.
  - \*7. Mathewson ND, Jenq R, Mathew AV, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol.* 2016;17: 505–513.
- Experimental study demonstrating that consortia of commensal clostridia protect against GvHD via the short chain fatty acid butyrate.**
- \*8. Swimm A, Giver CR, DeFilipp Z, et al. Indoles derived from intestinal microbiota act via type I interferon signaling to limit graft-versus-host disease. *Blood.* 2018;132:2506–2519.
- This proof of concept paper shows that a single bacterial tryptophan metabolite applied in the first days after experimental GvHD is able to increase survival from 10 to nearly 100% without affecting GvL.**
9. Peled JU, Devlin SM, Staffas A, et al. Intestinal microbiota and relapse after hematopoietic-cell transplantation. *J Clin Oncol.* 2017;35:1650–1659.
  - \*10. Bilinski J, Grzesiowski P, Sorensen N, et al. Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. *Clin Infect Dis.* 2017;65:364–370.
- 50 – 100% of hematological and HSCT patients receiving healthy donor FMT cleared colonization with multiresistant bacteria.**
11. Haak BW, Littmann ER, Chaubard JL, et al. Impact of gut colonization with butyrate-producing microbiota on respiratory viral infection following allo-HCT. *Blood.* 2018;131:2978–2986.
  - \*12. Taur Y, Coyte K, Schluter J, et al. Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant. *Sci Transl Med.* 2018;10:pii: eaap9489.
- An elegant randomized trial demonstrates restoration of a diverse microbiome by autologous FMT in HSCT patients.**
13. DeFilipp Z, Peled JU, Li S, et al. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. *Blood Adv.* 2018;2:745–753.
  - \*14. DeFilipp Z, Hohmann E, Jenq RR, et al. Fecal microbiota transplantation: restoring the injured microbiome after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2019;25:e17–e22.
- A comprehensive review on so far published data on fecal microbiota transplantation in HSCT recipients and its pathophysiological background.**
15. Hegazy AN, West NR, Stubbington MJT, et al. Circulating and tissue-resident CD4(+) T cells with reactivity to intestinal microbiota are abundant in healthy individuals and function is altered during inflammation. *Gastroenterology.* 2017;153:1320–1337.
  16. Connelly S, Bristol JA, Hubert S, et al. SYN-004 (ribaxamase), an oral beta-lactamase, mitigates antibiotic-mediated dysbiosis in a porcine gut microbiome model. *J Applied Microbiology.* 2017; 123:66–79.