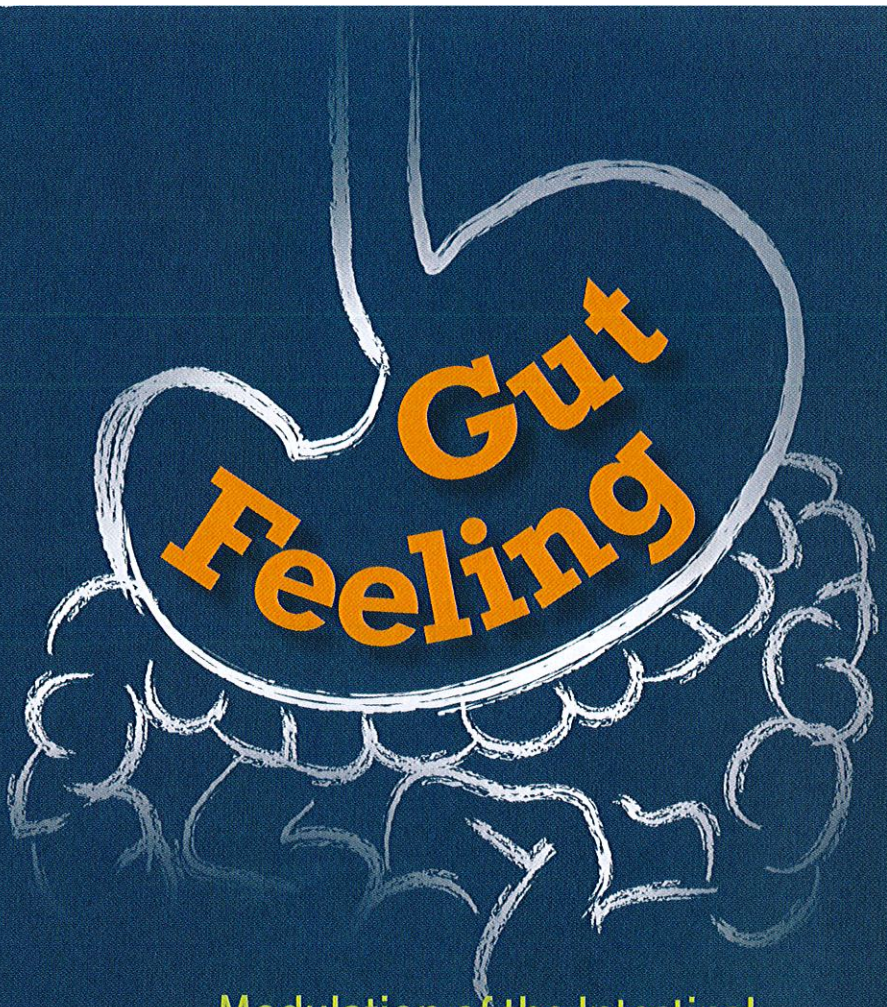


Modulation of the Intestinal Microbiome Could Provide Better Stem Cell Transplant Outcomes

By Kerri Wachter
AABB STAFF WRITER

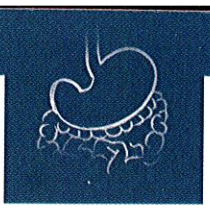
Not only has stem cell transplantation become a commonly used treatment for hematologic cancers and other diseases, it is under investigation as a potential treatment for a number of conditions ranging from multiple sclerosis to HIV to autoimmune disorders. Yet allogeneic stem cell therapy is still hampered by a number of complications, chief among them graft-versus-host disease (GVHD).

"The major outcome that is of interest to patients and bone marrow transplanters is graft-versus-host disease. That's one of the most common causes of morbidity and mortality after transplant," said Robert R. Jenq, MD, a medical oncologist at Memorial Sloan Kettering Cancer Center in New York. Roughly 8,000 allogeneic stem cell transplants (SCT) were performed in the United States in 2014 with GVHD accounting for 17 percent of deaths among individuals who underwent SCT from HLA-matched siblings from 2012 to 2013. Primary disease accounted for 48 percent and infection for 16 percent.

The common theme? How well the immune system is able to recover from the chemotherapy and radiation necessary to make the transplant successful.

Growing evidence points to a role for gut bacteria in immunologic recovery following stem cell transplantation. "When I think about the microbiome, I think about two things," said Ami Bhatt, MD, PhD, assistant professor of hematology and genetics at Stanford University. "First, how it can directly cause disease — and I think that only in the minority of cases do elements in the microbiome directly cause disease. But [second], I think much more often the microbiome modifies the disease phenotype. It makes things a little bit better or a little bit worse."

It's long been appreciated that intestinal bacteria in some way modulates SCT. "There's this old



understanding that intestinal bacteria are probably important in graft-versus-host disease but how that was happening and how to capitalize on it wasn't clear," said Jenq.

In the early 1970s, researchers found that germ-free mice — born and kept in isolation — had a reduced incidence of GVHD following SCT.[1] Others found that mice given antibiotics to eradicate intestinal bacteria also had very reduced or mild GVHD.[2] And in the 1980s, researchers demonstrated that protective isolation was associated with reduced GVHD and mortality in transplant patients.[3] However, others could not replicate these findings.

"There's more and more evidence that the immune system can be modified or modulated by gut bacteria," said Jenq. As it turns out, patients who have undergone bone marrow transplantation (BMT) may be an almost ideal population for studying gut bacteria because no other patient population has such heterogeneity. While the differences in gut bacteria among healthy individuals are somewhat subtle, with chemotherapy, antibiotics and changes in nutrition, many BMT patients often have radically altered gut bacteria. Yet other BMT patients can do well in terms of managing to eat well and developing fewer infections requiring fewer antibiotics.

A small community of researchers is now working to understand not only which bacteria are present in the gut microbiome, but how they are altered by stem cell therapy — including the use of antibiotics — and how these alterations affect the development of complications like GVHD and opportunistic infections, and ultimately mortality.

What's in the gut

Jenq and his colleagues have used statistical algorithms and a database of clinical outcomes from SCT patients to look at which bacteria or groups of bacteria are associated with better and worse outcomes.

In a 2015 study,[4] they evaluated fecal samples from 64 allogeneic blood/marrow transplant patients for bacterial composition. They found that patients with reduced GVHD-associated mortality had greater gut bacteria heterogeneity and in particular had increased levels of bacteria from the *Blautia* genus. They confirmed the association of greater levels of anaerobic *Blautia* with reduced GVHD-related mortality in 51 patients from the same institution. Greater levels of *Blautia* were also associated with greater overall survival. The loss of *Blautia* — a member of the *Clostridia* class — was associated with the use of anaerobic bacteria-inhibiting antibiotics and longer duration of parenteral nutrition.

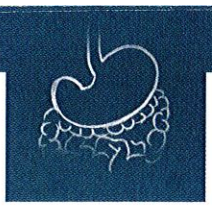
"It turns out that *Blautia* is really common in the human intestinal tract. It's one of the most common bacteria, and it's benign for the most part," Jenq noted. Moreover, there's a growing evidence that suggests that this bacteria is anti-inflammatory and beneficial in a variety of situations. In mouse models, bacteria like *Blautia* have been shown to increase anti-inflammatory regulatory T cells. There's also some evidence that a short-chain fatty acid produced by these bacteria — butyrate — mediates beneficial effects through several mechanisms. It can induce phosphate-3 expression, which is a transcription factor for regulatory T cells; it can also act as a nutrition source for the intestinal epithelium, according to Jenq.

Other bacteria appear to play a role as well. Using a different approach, Bhatt and her colleagues investigated umbilical cord hematopoeitic stem cell transplant (UC-HSCT) patients who developed cord colitis. "The whole point of this is that we know that our transplant patients — cord blood transplant patients in particular — are susceptible to unusual infections," she said.

In a study published in the "New England Journal of Medicine," Bhatt and her colleagues subtracted human and known bacterial DNA sequences from DNA derived from biopsy colon specimens from two patients who developed cord colitis following UC-HSCT. As a result, they were able to assemble a draft bacteria genome that had a very closely shared ancestry with genomes of Bradyrhizobium genus bacteria. Not only was the draft bacteria genome — tentatively named Bradyrhizobium enterica — also found in specimens from three other UC-HSCT patients with cord colitis, this bacteria species was not present in samples from healthy individuals.[5]

What it all means

There are several implications to understanding the role of certain bacteria in post-transplant complications or outcomes. First, it's possible that the gut microbiome could play a role in why some patients are able to better maintain nutrition and ward off infections.



"It may be that graft-versus-host disease is not just graft-versus-host disease, but is graft-versus-host's microbiota disease, because the donor and the recipient have different microbiomes," said Bhatt.

Jenq is working on a project with MSK colleague Doris Ponce, MD, to examine how much toxicity patients experience with preparatory chemotherapy. "There's heterogeneity there too, even though their all getting the same type of chemotherapy; it's possible that gut bacteria could play a role," he said.

Mouse models suggest this may be the case. "There we definitely know that the intestinal bacteria can change how toxic radiation is to the intestinal tract, for example. We have some early data that shows there's an effect there," Jenq said. "It's possible that bacteria could be changing how well people do with the transplant process."

Second, it may be possible to "load" a patient with beneficial bacteria in order to outgrow bacteria associated with poorer outcomes prior to transplant. It would also help in choosing more appropriate antibiotics should they be necessary. Lastly, this understanding could also aid the development of therapies should complications arise.

It may even be possible to develop a biomarker to indicate whether a patient is more susceptible to GVHD, infection or other poor outcomes. Such work is already underway. Ernst Holler, MD, PhD, and his colleagues at the University Medical Center in Regensburg, Germany, are investigating the use of low urinary indoxyl sulfate levels as markers of a disrupted intestinal microbiome. In a recent study, low levels of indoxyl sulfate — ultimately the result of degradation of the amino acid trypophan by intestinal bacteria — within the first 10 days after transplantation were associated with significantly greater transplant-related mortality in the first year, largely due to GVHD. If validated, indoxyl sulfate may provide a measure of GVHD risk in SCT patients.[6]

"We are not sure whether it [indoxyl sulfate] is sensitive enough to detect minor changes, which might be present at admission. However, we could clearly detect loss of diversity associated with use of antibiotics and GVHD," said Holler, who leads the allogeneic stem cell transplant unit and program in the department of hematology and oncology at the University Clinic Regensburg in Germany. "We are currently suggesting and testing IS as an early predictor of poor outcome in parallel to other serum biomarkers."

While indoxyl sulfate levels appear to be disturbed in the long term, the critical period appears to be the first 10 days, according to Holler. "We think that this period is the most vulnerable period, as the donor T cells come into a disturbed environment and thus do not have the conditions [such as butyrate-rich environment] that they would need to maintain immunological tolerance in the gut," Holler said. This early time period following transplant may be akin to the the neonatal period and the first year when the immune system is developing. "If the first days of donor cell infiltration occur in a diverse setting, later disturbances are less relevant," he said.

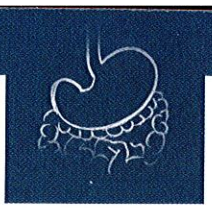
They also found that high urinary levels of indoxyl sulfate were associated with the presence of high levels of two members of the Clostridia class: Lachnospiracaea and Ruminococcacaea. Low levels were associated with members of the Lactobacillales order (of the Bacilli class). These results are in line with previous research[7], in which they found *Enterococcus faecium* and *Entercoccus gilvus* (members of the Lactobacillales order) more frequently following transplant, particularly in patients with GVHD.

Antibiotics

"The other potential ramification of our work," Jenq noted, "is to pick antibiotics in a smarter way. So not only do you want to prevent or treat infections, but maybe you want to pick antibiotics that will spare your good bacteria and that can help improve outcomes."

In the NEJM study, Bhatt and colleagues also found that specimens from the original UC-HSCT two patients with cord colitis showed greatly reduced levels of *B. enterica* following antibiotic administration compared with specimens collected prior to antibiotics.

Jenq and his coinvestigators have identified specific antibiotics that could play a role in GVHD-associated mortality in an unpublished paper. The findings suggest another mechanism for how the gut microbiome might affect SCT outcomes. They started by analyzing the antibiotics received by different patients (selected largely because of drug allergies). SCT patients who received piperacillin/tazobactam and imipenem had a much greater GVHD-associated mortality rate. Patients who received



cefepime and aztreonam did not have greater rates of GVHD-associated mortality.

They tested these four antibiotics in a mouse model of GVHD and found the same levels of severity. Mice that received imipenem and piperacillin/tazobactam did worse and those that received cefepime and aztreonam did better. They found that it was specifically colon GVHD that was different between the two groups, while liver and skin GVHD were not changed by the antibiotics.

When the researchers examined this further, they found that mice treated with imipenem lost *Clostridia* (the class to which *Blautia* — and Lachnospiracaea and Ruminococcacaea — belongs) in their colon, but had an outgrowth of *Akkermansia*. This genus of bacteria can digest mucus, using it as a source of carbohydrates. These mice had no mucus layer in the gut. "I think that's another mechanism. Bacteria like *Blautia* and other Clostridia, may help prevent the overgrowth of other bacteria that could be harmful, such as a mucus-eating bacteria," Jenq said.

Work like this "will make us think very carefully about the risks and benefits of exposing patients to broad-spectrum antibiotics," said Bhatt. "As transplantation physicians we tend to use antibiotics willy nilly. And certainly antibiotics are incredibly effective in improving mortality in our sickest patients, but there is a cost associated with exposure to broad-spectrum antibiotics. We need to have a little more restraint when using them because we may be exposing our patients to short-term gains but long-term harm."

Modifying the gut microbiome

A greater understanding of the gut microbiome and its relationship with SCT outcomes could ultimately lead to better preparing or treating transplant patients to modify their gut bacteria, which could lead to better outcomes. "I do think that we'll make some impact in terms of graft-versus-host disease, as well as idiopathic problems that our patients face," said Bhatt.

It might also be possible to give patients a pre- or probiotic after they've completed their chemotherapy to reconstitute their intestinal bacteria. "We're interested in being able to tune the microbiome," said Bhatt.

The researchers are experimenting with the use of prebiotics — typically carbohydrates that are only digestible by certain microbes. The idea is to encourage the growth of certain beneficial organisms that will outcompete pathogenic or harmful bacteria.

Another possibility is to collect stool from a patient before chemotherapy, freeze it and give it back to them. This is comparable to preoperatively collecting a patient's blood and giving it back to them during or after surgery. Jenq and his group are investigating the use of such enemas.

Bhatt's group is also interested in deep molecular characterization of what happens after patients receive fecal microbiota transfer from healthy individuals. It isn't clear when this fecal transfer should occur — before, during or after SCT. "One of the things that we've been thinking about is doing fecal microbiota transfer at the time of stem cell transfer. Ideally we'd give patients fecal microbiota transfers from their donor," she said.

"A key thing to consider for the future is that the microbiome is closely tied to immunologic development," Bhatt said. There is reason to believe that the microbiome may be related to immunologic reconstitution in the setting of transplantation. "It may be that by tuning the microbiome, we can improve immune reconstitution," she said.

ENDNOTES:

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