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The microbiome and immune regulation after transplantation

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Abstract

The trillions of microorganisms inhabiting human mucosal surfaces participate intricately in local homeostatic processes as well as development and function of the host immune system. These microorganisms, collectively referred to as the “microbiome”, play a vital role in modulating the balance between clearance of pathogenic organisms and tolerance of commensal cells, including but not limited to human allografts. Advances in immunology, gnotobiotics, and culture-independent molecular techniques have provided growing insights into the complex relationship between the microbiome and the host, how it is modified by variables such as immunosuppressive and antimicrobial drugs, and its potential impact on posttransplantation outcomes. Here we provide an overview of fundamental principles, recent discoveries, and clinical implications of this promising field of research.

INTRODUCTION

The “microbiome” embodies a biological ecosystem that encompasses the collective ensemble of bacteria, viruses, fungi, and other microorganisms (ie microbiota) together with their metabolites, degradative byproducts, and genomes, as suggested by the Greek root words “*bios*” (life) and “*oma*” (mass). Although frequently referred to in the context of the digestive tract (Figure 1), in particular the colon, various other regions of the body also

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- made substantial contributions to the conception and design of the work
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foster the microbiome, including the respiratory tract, oral cavity, vagina, conjunctiva, and skin. Throughout human life, the microbiome participates intricately in local as well as systemic homeostatic processes, including an increasingly recognized modulatory role in the development and function of the host immune system. It follows, thus, that the microbiome may have a significant impact on tolerance and survival of transplanted allografts and transplant recipient outcomes; indeed, this putative relationship has become an area of growing biomedical intrigue, catalyzed further by methodological (eg –omic and deep sequencing techniques) advances. Moreover, insofar as allografts themselves harbor immune cells and/or effectuate immunobiological activities, they may reciprocally affect the shape, composition, and function of the human microbiome.^(1, 2)

While current understanding of these complex, multidirectional dynamics is incomplete, their potential implications have piqued broad and growing interest and are a subject of increasing basic, translational, and clinical research. In this review, we provide a synopsis of fundamental principles, recent insights into, and clinical applications of the intriguing relationship between the microbiome and immune regulation in the context of transplantation.

MICROBIAL RECOGNITION: THE HOST INNATE IMMUNE SYSTEM

Vital to host recognition of microorganisms (symbionts and pathobionts) are pattern-recognition receptors (PRRs). PRRs, which include toll-like receptors (TLRs) and nucleotide-binding oligomerization domain receptors (NLRs),^(3–5) are plasma membrane and/or intracytoplasmic proteins that sense and respond to pathogen-associated molecular patterns (PAMPs) such as microbial lipid, protein and nucleic acid moieties⁽⁶⁾ as well as microbial metabolites (eg bile acids,⁽⁷⁾ short-chain fatty acids,⁽⁸⁾ and phosphatidylcholine.^(7, 9)

PRRs become activated when bound by their cognate PAMPs, be they from the arterial or venous circulation, intestinal, bronchial, or biliary lumen, or vaginal cavity. This induces signal transduction cascades which involve recruitment of adaptor proteins and activation of a multitude of downstream signaling molecules, thus culminating in: i) nuclear translocation of transcription factors, ii) biosynthesis of interleukins, interferons, and/or chemokines, eg tumor necrosis factor (TNF- α) and interleukin (IL-1 β), and iii) regional and recruited cell responses, including those of the adaptive immune system (Figure 1).⁽⁵⁾ NLRs function similarly to and often in conjunction with TLRs.

Given the intricate and far-reaching nature of PRR signaling, it is not surprising that its dysfunction is associated with a variety of infectious, autoimmune, and metabolic disorders and may impact allograft and host health.⁽¹⁰⁾

THE ADAPTIVE IMMUNE SYSTEM

The adaptive immune system is composed of highly specialized cells and processes which generate immunological memory after exposure to an antigen. The major cellular constituents of the innate immune system are B and T lymphocytes, which are discussed further below in relation to immunoregulation.⁽¹¹⁾

T lymphocytes and immune regulation: central and peripheral tolerance

Development of T lymphocyte tolerance is generally the primary means of immunotolerance in humans. This occurs via 2 main mechanisms: central tolerance and peripheral tolerance. Central tolerance includes elimination of intrathymic self-reactive clones (ie T lymphocytes with receptors [TCRs] recognizing self-antigens) during maturation.⁽¹²⁾ Peripheral tolerance refers to elimination of peripherally activated T lymphocyte clones in the periphery and is the primary mode of tolerance to transplanted organs and cells. It occurs through clonal deletion via either T lymphocyte apoptosis, development of T lymphocyte anergy, and/or suppression of alloreactive T lymphocytes by regulatory cells and inhibitory cytokines, each of which are addressed hereinafter.⁽¹³⁾ Briefly, T lymphocyte apoptosis is induced via 2 pathways:⁽¹⁴⁾ i) death receptor activation, which occurs following antigenic stimulation of mature T lymphocytes and ii) loss of growth factors (eg IL-7, IL-15) with a shift toward a proapoptotic milieu.⁽¹⁵⁾ With regard to anergy, it is a process that can be conceptualized as a quality-control mechanism through which lymphocyte expansion/activity is inhibited; it provides the benefit of (allograft) tolerance without suppressing the immune system as a whole.

IMMUNOREGULATORY CELLS AND TOLERANCE POST-TRANSPLANTATION

The concept of acquired cellular and humoral tolerance to nonhost cells was first recognized decades ago.⁽¹⁶⁾ Among the most central of these are regulatory T lymphocytes (Tregs), but others are also noteworthy, as summarized below and discussed in detail elsewhere.⁽¹⁷⁾

Tregs: highlights and relevance in transplantation

Tregs play a critical role in posttransplant tolerance induction and prevention of graft rejection. The 2 main types of Tregs are: i) naturally-occurring thymus-derived Tregs, which are anergic to TCR stimulation and can suppress proliferation of other T cells,⁽¹⁸⁾ and ii) inducible Tregs, which develop peripherally from naïve T cells with acquisition of Foxp3 expression and in the setting of chronic antigenic stimulation and transforming growth factor (TGF)- β 1.⁽¹⁹⁾ Tregs suppress effector T cells through secretion of inhibitory cytokines (eg IL-10), interaction with CD80/CD86 through CTLA-4,⁽²⁰⁾ and functioning as cytotoxic T cells which destroy activated CD4⁺ and CD8⁺ T lymphocytes via perforin-dependent mechanisms.⁽²¹⁾

In preclinical models, the enhancement of CD4⁺CD25⁺ T cells prevents allograft rejection in an IL-10 and CTLA-4 dependent manner;⁽²²⁾ likewise, depletion of CD25⁺ T lymphocytes results in accelerated rejection of allogeneic skin grafts and increases graft-versus-host disease (GVHD)-related mortality.⁽²³⁾ Results of preliminary studies investigating the role of immunosuppressive properties of Tregs in GVHD appear promising.

Natural Killer T lymphocytes (NK-Ts)

NK-Ts constitute less than 1% of all circulating lymphocytes but participate in critical cellular processes and signaling. NK-Ts and Tregs engage in crosstalk by secreting IL-4 and IL-10, respectively, which leads to immunotolerance by activating negative costimulatory

pathways such as PD1/PD-L1.⁽²⁴⁾ NK-Ts are protective against GVHD in hematopoietic stem cell transplantation (HSCT) recipients and induce immunotolerance in solid organ transplantation recipients.⁽²⁵⁾ Pretransplantation total lymphoid irradiation increases NK-Ts posttransplantation and results in mitigation of (untoward) immune responses. NK-Ts expanded *ex vivo* can be used as an adjunctive therapy to enhance graft-versus-tumor activity and induce tolerance.⁽²⁶⁾

Regulatory B lymphocytes (Bregs)

A small subset of the B lymphocyte population has immunosuppressive activities that can be observed after solid organ transplantation. Human Bregs express CD19, CD24, and CD38, secrete IL-10 (a known regulator of immune responses), and may promote the conversion of naïve T lymphocytes to inducible Tregs.⁽¹⁷⁾⁽²⁷⁾ Bregs transiently appear in the peripheral blood of kidney transplant recipients after alemtuzumab induction, prior to naïve B lymphocyte repopulation.⁽²⁸⁾ Increased Tregs are also found after alemtuzumab induction, suggesting coordinated activity with Bregs posttransplantation.⁽²⁹⁾ In addition, higher numbers of naïve and transient B lymphocyte populations have been found in peripheral blood of tolerant versus nontolerant kidney transplant recipients;⁽³⁰⁾ this supports the role of Bregs in tolerance, but the mechanism of their activation interaction with Tregs remain uncertain.

Tolerogenic Dendritic cells (tDCs)

Constituting a small subset of DCs, tDCs induce immunotolerance via promotion of T lymphocyte anergy and Treg activation. Phenotypically, they are immature DCs that have a diminished ability to produce inflammatory cytokines (eg IL-12) and express low level of MHC class II and costimulatory molecules (CD80 and CD86).⁽³¹⁾ Infusion of bone marrow- or monocyte-derived immature DCs enhances allograft survival in murine models.^(32, 33) Plasmacytoid DCs (pDCs) are another subset of DCs that express cell-surface CD4, MHC class II, and CD123, secrete high amounts of type I interferons and TNF- α , and can induce tolerance through Treg interactions.⁽³⁴⁾ Bone marrow-derived CD8+TCR- facilitating cells (FCs) are also a plasmacytoid-precursor DC subpopulation that induces Tregs and enhances HSCT engraftment.⁽³⁵⁾ FCs are activated via TLR-9 signaling and expanded by Flt-3 ligand. Administration of FCs has been recently reported to induce tolerance in combined kidney-HSC transplant recipients.⁽³⁶⁾

THE MICROBIOME AND ITS ROLE IN HOST IMMUNE REGULATION

It has become increasingly evident over the last decade that the human microbiome participates integrally in eliciting and regulating host immunobiological responses. Several lines of evidence support the notion that this relationship is in fact reciprocal, begins perinatally, and is crucial for tissue (eg mucosa-associated lymphoid tissues) and organ development.^(1, 37, 38) Differentiation of human myeloid cell progenitors has been shown to be suppressed in germ-free animals, thus suggesting that the gut microbiome directs hematopoiesis differentiation and innate immune development.⁽³⁹⁾ The gut microbiome has also been shown to potentially play a role in protecting against autoimmunity; inoculation of

mice with defined clostridial strains was found to elicit an immune response resulting in the accumulation of Tregs and resistance to colitis.⁽⁴⁰⁾

The orchestrating role of the microbiome in T lymphocyte differentiation, in particular, is of key importance in the setting of transplantation. In murine models, it has been shown that segmented filamentous bacteria (a group of gram-positive organisms related to clostridia) drive T lymphocyte differentiation into Th17 and Th1 cells in the lamina propria as well as systemically.⁽⁴¹⁾⁽⁴²⁾ Various other examples of this concerted regulatory role on host innate and adaptive immunity have been described, as summarized in Figure 1.

While research has mostly focused on the role of the *intestinal* microbiota in immune homeostasis, it has been shown that commensal microbiota of other mucosal surfaces participate in immune regulation as well. For example, the commensal respiratory tract microbiota appears to play a role in T lymphocyte priming and migration of dendritic cells from the lung to draining lymph nodes.⁽⁴³⁾ *Lactobacillus* inoculation of the nasal passages in mice was recently found to elicit an immune response characterized by infiltration of neutrophils and cytokine secretion; this protected mice against lethal pneumovirus infection.⁽⁴⁴⁾

It should also be noted that metabolites of the microbiota, eg short chain fatty acids such as butyrate and acetate, appear to be key contributors in regulating the gut microbiota-epithelium interaction. These fatty acids interact with metabolite sensing G protein-coupled receptors on endothelial cells and help to maintain gut homeostasis.^(45–47)

Advances in methods used to study the microbiota

Microbiology has long relied on culturing of microorganisms as the primary research tool. However, microscopic analyses of human feces have shown that 60–80% of the bacteria cannot be cultured.⁽⁴⁸⁾ Using multi-locus sequence typing (MLST), fragments from eleven housekeeping genes can be amplified and sequenced to classify bacterial species.⁽⁴⁹⁾ While MLST is useful for characterizing several organisms, it is limited by the inability to recognize organisms lacking sequences for MLST. Use of 16s rRNA sequencing can overcome this limitation, although this approach is limited by biases resulting from copy number variations and identification of closely related species.⁽⁵⁰⁾ High-throughput shotgun screening was recently developed and seems overcome this drawback. Here, a genome databases is created for each newly isolated organisms;⁽⁵¹⁾ while this is a relatively accurate method for identifying microbial components and has been increasingly applied in recent years, it requires significant computational analyses and is time-consuming. These and other techniques are being widely incorporated into microbiome research, continue to evolve, and are increasingly moving to clinical study applications.

Another consideration and challenge in studying the human microbiome is the dynamic nature of its constituents and the observation that there are quantitatively scant (i.e. difficult to isolate and culture) yet qualitatively substantive organisms. Regarding the former, as summarized in Figure 2, the content and function of the microbiome can be affected directly or indirectly by chemotherapeutic and immunosuppressive drugs, antimicrobial treatment and prophylaxis, inflammatory processes secondary to ischemia/reperfusion and

allorecognition, the underlying cause of organ failure (i.e. the original indication for transplantation), anatomical alterations secondary to surgical intervention (e.g. resection, anastomoses), and host genetics. How the ensuing alterations and dysbiosis occur and affect alloresponses represents an exciting field of biomedical research.

ALLOGRAFT REJECTION, TOLERANCE, POSTTRANSPLANT COMPLICATIONS, AND THE MICROBIOME

Solid organ transplantation

The increasingly recognized bidirectional interaction of human microbiota with the host and its immune system (Figure 2) has sparked significant interest given its potential impact in posttransplantation outcomes, including the prospect for generating novel preventative and therapeutic strategies to promote immunotolerance and mitigate allograft rejection and GVHD.

With respect to liver transplantation, investigators from China collected fecal and blood samples from 111 liver transplant recipients in China and found significant alterations in gut microbiota, including lower levels of Eubacteria, *Bifidobacterium*, *Faecalibacterium* and *Lactobacillus* and higher levels of *Enterococcus* and Enterobacteriaceae in liver transplantation recipients; except for *Enterococcus*, all bacteria returned to normal levels 6 months posttransplantation.⁽⁵²⁾ In another study, decreased microbial diversity posttransplantation was associated with increased risk of postoperative infections.⁽⁵³⁾ With respect to the microbiome and the underlying disease (eg indication for liver transplantation), a recent report described successful treatment of recurrent primary sclerosing cholangitis, an idiopathic disease with emerging links to microbial dysbiosis.^(5, 54, 55) Variation of gut microbiota was found to predict early acute cellular rejection after liver transplantation in a rat model,⁽⁵⁶⁾ suggesting that a microbiota profile or alterations therein might hold promise as a predictor of acute rejection posttransplantation. Additionally, gut microbiota have been linked to the development of new-onset diabetes mellitus postliver transplantation through alterations of host metabolic homeostasis by as yet unclear gut-liver axis mechanisms.⁽⁵⁷⁾

In kidney transplantation, a recent pilot study was undertaken wherein comprehensive analysis of oral, urine, and stool microbiota was performed before as well as at 1 and 6 months after transplantation; this revealed substantial changes after transplantation that persisted over time.⁽⁵⁸⁾ In addition, these changes were associated with acute rejection, diarrhea, and urinary tract infection rates posttransplantation, with each complication having its own microbiological correlate (eg a lower Shannon diversity index posttransplantation being a predictor of diarrhea).⁽⁵⁹⁾

Lung transplantation recipients also appear have alterations in the pulmonary microbial community based on analyses of bronchoalveolar lavage (BAL) specimens from 21 patients. In 1 study, lung transplant recipients have higher bacterial burden in BAL specimens than control subjects, with frequent appearance of dominant organisms and decreased microbial diversity.⁽⁶⁰⁾ In another study, the microbial communities in BAL specimens from 57 lung

transplantation recipients were characterized and correlated with rates of bronchiolitis obliterans, a major complication of allograft rejection. Reestablishment of pretransplant pulmonary microorganisms appears to protect against bronchiolitis obliterans.⁽⁶¹⁾

Similar to other organs, dysbiosis is associated with increased rates of rejections and inferior outcomes in the case of small intestinal transplantation. Indeed, among small intestinal transplant recipients who experienced acute rejection, Firmicutes and Lactobacillales were significantly reduced while Proteobacteria were increased in ileal effluents; the overall findings suggested that microbiota profiling may serve as a potential diagnostic biomarker for risk of small intestinal allograft rejection.⁽⁶²⁾

The aforementioned findings suggest that monitoring the gut microbiome peritransplantation can provide a potentially useful surrogate marker for outcomes posttransplantation, and manipulation of the microbiome may help realize improved outcomes in this regard.

Hematopoietic stem cell transplantation (HSCT)

HSCT has emerged as a potentially curative therapy for various hematological malignancies as well as nonmalignant diseases. Despite improved outcomes post- HSCT over the last decade, GVHD and disease relapse continue to be major problems. Several studies have shown that alterations of the enteric microbiome play an important role in outcomes post-HSCT. In 1 study, fecal specimens were collected from 80 HSCT recipients and were classified into high, intermediate, and low diversity groups according to Bacterial 16S rRNA sequences.⁽⁶³⁾ Mortality was significantly higher in patients with lower intestinal diversity (64% vs 33% for the low and high diversity group, respectively). Changes in the microbiome post-HSCT are also associated with subsequent bacteremia, and fecal microbiota characteristics (eg domination by Proteobacteria and *Enterococcus*) appear to predict patients at greatest risk.⁽⁶⁴⁾ In a study published this year, abundance of *Eubacterium limosum* was found to be associated with less relapse, while *Enterococcus* was associated with increased rates of relapse post-HSCT.⁽⁶⁵⁾ Another study using next-generation sequencing of the V3 hypervariable region of the 16s rRNA gene on serial stool specimens in patients receiving HSCT observed a relative shift toward Enterococci post-transplantation.⁽⁶⁶⁾

The cross-talk between the microbiome and the host immune system has also been evident in recent studies which showed that alterations of the microbiome increased tumor infiltration lymphocytes and affected response to checkpoint inhibitors and adoptive T cell therapy. In melanoma murine models, transplantation of microbiota with higher anti-tumor activity augmented T lymphocyte response and inhibited tumor growth after PDL1 inhibition; the investigators were able to identify *Bifidobacterium* as the bacterium responsible for this, suggesting that altering the amount of *Bifidobacterium* in humans can enhance anti-PDL1 treatment efficacy.⁽⁶⁷⁾ In a similar vein, *Bacteroides* has been found to have a key role in the immuno-stimulatory effect of CTLA-4 blockade; tumors in antibiotic-treated mice did not respond to CTLA blockade, while the adoptive transfer of *B. fragilis*-specific T lymphocytes resulted in significant CTLA-4 mediated anti-tumor effects.⁽⁶⁸⁾

The microbiome and GVHD

There is growing evidence that changes in the microbiome are linked to the pathogenesis and outcomes of GVHD. GVHD and mucosal inflammation are both associated with major shifts in the microbiota in human and mouse HSCT recipients. Furthermore, in murine models of GVHD, eliminating *Lactobacillus* pre-HSCT aggravated GVHD, whereas reintroducing it mediated significant protection against GVHD.⁽⁶⁹⁾ In an effort to uncover the mechanisms of this process, investigators have studied the effect of GVHD on Paneth cells and found that Paneth cells are targeted by GVHD;⁽⁷⁰⁾ this results in a marked reduction in expression of α -defensins, which selectively kill noncommensal organisms. Molecular profiling showed overwhelming expansion of *E. coli* and subsequent bacteremia in these models. A recent study found increased bacterial diversity and harboring increased amounts of *Blautia* to be associated with reduced GVHD mortality and improved survival.⁽⁷¹⁾ Lastly, a study published this year found that fucosyltransferase 2, a genetic modifier of the human microbiome, influences risk of GVHD.⁽⁷²⁾ With respect to other components of the microbiome, transfer of lipopolysaccharide (LPS) into the systemic circulation has been found to be crucial to the induction of cytokine dysregulation contributing to GVHD. Antagonism of LPS in a murine transplant model reduced GVHD and significantly improved survival compared to controls and had no deleterious effect on donor T lymphocyte responses.⁽⁷³⁾

To ameliorate the dysbiotic microbiome and prevent or treat GVHD, the use of antibiotics and probiotics has been investigated. In murine models, administration of polymyxin B inhibits *E. coli* overgrowth and ameliorates GVHD.⁽⁷⁰⁾ In a study of 112 pediatric patients undergoing HSCT, total gut decontamination with high-dose nonabsorbable antimicrobials was successful in approximately 50% of children, with the degree of decontamination correlating with occurrence and severity of GVHD.⁽⁷⁴⁾ It should be noted that in addition to antibiotics and probiotics, healthy donor fecal transplant may represent a durable therapeutic intervention on the microbiome and transplant-related disease, as already demonstrated in infectious human disease.⁽⁷⁵⁾

SUMMARY

Human immune responses are tightly regulated via coordination of myriad cells and signaling molecules and constant exposure to self and foreign antigens. In this regard, the microbiome plays a vital role in cellular crosstalk with numerous cell types and in coordinating immune responses within the host, and reciprocally, is under constant stimuli from the host, be this cells, cytokines, antibiotics, xenobiotics, or other. This dynamic relationship, though incredibly complex, has the potential to be harnessed as part of therapeutic strategies to induce immunotolerance and decrease post-transplantation complications. Shortcomings and limitations of current techniques are many, including challenges in designing and executing methodologically rigorous microbiome studies (eg due to monetary and temporal costs, animal and microbial strain phenomena, etc.), establishing the directionality of microbiome and host changes posttransplant, and distinguishing between cause, effect, and coincidental association. We anticipate that within the next decade, study of the human microbiome will become increasingly elegant, efficient,

and cost-permissive. Ultimately, a central goal in this context is safe, efficient, and effective assaying and manipulation of the microbiome—likely through a variety of pharmacologic and other minimally-invasive means (eg fecal transplant)—to better predict and enhance outcomes following solid organ and HSC transplantation.

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ABBREVIATIONS PAGE

BAL	bronchoalveolar lavage
Bregs	B regulatory lymphocytes
FCs	CD8+TCR- facilitating cells
GVHD	graft-versus-host disease
HSCT	hematopoietic stem cell transplantation
IL	interleukin
IFNs	interferons
LPS	lipopolysaccharide
MHCs	major histocompatibility complexes
NK-Ts	Natural Killer T lymphocytes
NLRs	nucleotide-binding oligomerization domain receptors
PAMPs	pathogen-associated molecular patterns
pDCs	Plasmacytoid dendritic cells
PRR	pattern-recognition receptors
TGF-β	transforming growth factor β
TCRs	T cell receptors
TLRs	toll-like receptors
TNF-α	tumor necrosis factor α
Tregs	T regulatory lymphocytes

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Figure 1.

Conceptual schematic of the multifaceted interaction between the enteric microbiome and the innate and adaptive human immune system. Luminal microorganisms express or metabolize a variety of molecules that can bind to and be recognized by pattern-recognition receptors PRRs such as toll-like receptors (TLRs) and NOD-like receptors (NLRs) on epithelial and other host cells. TLRs signal through MyD88 and MyD88-independent mechanisms and activate a series of signal transduction pathways which culminate in recruitment of effector cells, upregulation of costimulatory molecules on antigen presenting cells (D), enhancement of antigen presentation to T lymphocytes (D), and increased biosynthesis of cytokines and other signaling mediators. Segmented filamentous bacteria (a group of gram positive organisms related to clostridia) drive TH17 and TH1 differentiation (A, B) and compete with pathogenic microbes (eg *S. enteritidis*) in the intestinal tract. Various clostridial species promote expansion of Tregs with increased levels of TGF-β and IL-10 (C). Polysaccharide A from *Bacteroides* organisms has been shown to drive the differentiation of TH2 to TH1 T lymphocytes (A), enhance proliferation of Tregs, increase production of IL-10, and protect against experimental colitis (C). Short chain fatty acids (SCFAs), which are bacterial fermentation products of human ingesta, stimulate Treg proliferation, and help regulate Treg homeostasis (C). The enteric microbiota are a source of peptidoglycan that primes the innate immune system and enhances neutrophil function (eg bactericidal activity) via NOD1 NLR signaling (F). IgA from the intestinal lumen is transcytosed into the cytoplasm and plays a critical role in mucosal immunity as a first-line defense mechanism (E). Analogous molecular and cellular signaling occurs throughout other constituent anatomical regions of the human microbiome (eg respiratory tract, skin) with local and systemic implications.

Figure 2.

Diagram of dysbiotic alterations and associated host- and graft-level sequelae. Several factors pre-, intra-, and/or posttransplantation can result in an altered microbiome and consequent dysbiosis. As shown in this figure, these include use of antimicrobials and immunosuppressant drugs, chemotherapy (eg high-dose conditioning for hematopoietic stem cell transplantation [HSCT]), and the new post-surgical anatomy (which can pose anatomical as well as functional/neuromuscular changes). Dysbiosis may lead to or modify several posttransplantation complications such as risk of infection (urinary tract infection, infectious diarrhea), adverse immunologic phenomena (bronchiolitis obliterans postlung transplantation, autoimmune hemolytic anemia), graft-versus-host disease (post- HSCT), graft rejection, recurrence of underlying disease (eg recurrent primary sclerosing cholangitis post-liver transplantation), and increased mortality rates. Selectively avoiding these alterations and inducing eubiotic changes in the peri-transplantation setting may hold preventative and therapeutic potential.