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Reviews

Emerging Influence of the Intestinal Microbiota during Allogeneic Hematopoietic Cell Transplantation: Control the Gut and the Body Will Follow

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A B S T R A C T

The intestinal microbiota has many critical roles in maintaining gastrointestinal epithelial and gastrointestinal systemic immune homeostasis. This review provides insight into how allogeneic hematopoietic cell transplantation (HCT) and its associated complications and supportive care therapies affect the microbiota. Additionally, the review discusses how preservation and restoration of the microbiota might be advantageous in decreasing HCT-related morbidity and mortality.

INTRODUCTION

For over 40 years, researchers have known that the commensal bacteria inhabiting our intestines, collectively termed the intestinal microbiota, are important modulators of the biology of hematopoietic cell transplantation (HCT). Clinical studies using strategies to suppress the intestinal microbiota to prevent acute graft-versus-host disease (GVHD) showed considerable promise early on but eventually failed to demonstrate consistent benefit. The concept of manipulating the microbiota to improve outcomes for patients was not forgotten, but the best means of doing so has remained unclear.

Over the years, advances in culture-independent techniques have led to an enhanced understanding of bacterial subtypes beyond conventional microbiologic culture techniques. Sequencing of the 16S rRNA gene was developed in the 1980s as a powerful phylogenetic tool [1]. Coupled with subsequent genetic methodologies, including in situ hybridization and PCR, 16S characterization allows rapid identification of bacterial isolates, including potential pathogens from clinical samples [2]. A more recent major advance has been the advent of high-throughput sequencing modalities, or "deep sequencing" methods, that allow characterization of the composition of mixed bacterial samples. Deep sequencing of 16S rRNA has yet to be used routinely in the clinical setting because of practical barriers to implementation, including time, cost, and complexity, although newer, more rapid techniques are being developed [3].

A role for the microbiota has thus been re-examined in relation to a variety of clinical outcomes, ranging from obesity and atherosclerosis, to allergies and asthma, and

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even to cancer development and autism. HCT has been no exception, with several groups recently uncovering important relationships between the microbiota and outcomes in HCT recipients. In this review we provide an update regarding our knowledge of the biology of intestinal homeostasis in relation to the microbiota and a summary of recent findings implicating cross-talk between the microbiota and intestinal immunity that appears to have a significant impact on outcomes after HCT.

INTESTINAL EPITHELIAL HOMEOSTASIS

The human gastrointestinal (GI) tract can harbor an estimated 100 trillion individual bacteria belonging to roughly 1000 species in any single individual, and 15,000 species of bacteria have been identified from human GI samples [4]. These bacteria exist in a symbiotic relationship with their host, and thus a critical function of the intestinal mucosa is to enforce immunologic tolerance to these bacteria while maintaining the potential to mount protective responses against pathogens when necessary.

The intestinal epithelium, composed of intestinal epithelial cells (IECs) connected by tight junctions, creates a physical and biochemical barrier to separate luminal organisms from intestinal tissues [5]. Intestinal epithelium is organized into small finger-like projections called villi, and in between villi are tubular invaginations called crypts. Intestinal epithelial stem cells, which give rise to all subsets of differentiated IECs, reside at the base of these crypts and are dependent on Wnt signaling. They can develop into specialized secretory IECs, including goblet cells, enteroendocrine cells, enterocytes and Paneth cells [6]. Goblet cells secrete mucins into the intestinal lumen to form a mucous layer that acts as a first line of defense against microbes [7]. Paneth cells are specialized cells found within the crypts that secrete a variety of antimicrobial peptides, including defensins, cathelicidins, and calprotectins. These have activity against a variety of microbes and function by compromising the integrity of microbial cell membranes [8]. A recently described antimicrobial peptide, the C-type lectin regenerating islet-derived protein III gamma (RegIIIγ), is secreted by enterocytes in a MyD88-dependent manner and plays an important role in separating luminal bacteria from the intestinal epithelial surface [9,10].

The intestinal mucosa is in contact with harmless commensal bacteria as well as potential pathogens, thereby functioning to immunologically survey the intestinal lumen [11]. IECs monitor the tone of the lumen, recognizing a variety of microbial products in both antigen-independent and antigen-dependent manners to participate in coordinated immune tolerance or, alternatively, immune response [8]. IECs express pattern-recognition receptors, including Toll-like receptors, nucleotide-binding oligomerization domain (NOD)-like receptors, and retinoic acid-inducible gene 1 (RIG-I)-like receptors, for the recognition of microbial ligands or endogenous signals associated with pathogenesis to modulate cellular responses [12,13]. A subpopulation of IECs called microfold cells mediate sampling of antigens and microorganisms for presentation to the mucosal immune system [14]. Subepithelial macrophages also sample luminal contents through transepithelial dendrites [15]. Dysregulation of the surveillance mechanisms used by the intestinal mucosa can lead to the development of inflammatory bowel disease and other disorders [16].

Interestingly, many aspects of intestinal immune homeostasis fail to develop in the absence of the intestinal microbiota. Germ-free mice, housed in sterile conditions, show extensive defects and impaired development of gut-associated lymphoid tissues and antibody production [17]. IECs in germ-free mice have altered patterns of microvilli formation and decreased rates of cell turnover compared with wild-type, leading to defective expansion of defensins and other antimicrobial peptides.

A variety of hematopoietic cells participates in intestinal homeostasis. Myeloid cells, including macrophages and dendritic cells (DCs), are important mediators of both tolerance and protection. Nonmigratory macrophages maintain close contact with IECs to phagocytose and mediate clearance of pathogens and commensal bacteria that traverse the epithelial barrier [18]. In response to commensal bacteria, IECs produce cytokines via pattern-recognition receptor signaling, promoting the development of DCs and macrophages with tolerogenic properties [8], including specialized transforming growth factor-β–producing $CD103^{+}CD11b^{+}$ DCs within the GI tract that can induce forkhead box P3 (Foxp3)-expressing regulatory T cell (Treg) expansion [19]. Upon recognition of bacteria, DCs carry antigenic material and live bacteria to secondary lymphoid tissues, including mesenteric lymph nodes and Peyer's patches, and present them to adaptive immune cells [18]. This induces the differentiation of Tregs as well as gut-homing properties on T cells for the recruitment of recirculating mature cells to the original site of antigen encounter at the intestinal lamina propria [20]. Indoleamine 2,3-dioxygenase (IDO), an enzyme expressed by DCs and macrophages, catalyzes the initial rate-limiting step in tryptophan degradation. IDO is induced during inflammation by inflammatory cytokines, such as IFN-γ, and inhibits T cell activation through the consumption of tryptophan and subsequent expansion of Tregs, aiding in intestinal homeostasis [21]. Germ-free mice exhibit reduced levels of IDO, suggesting a role of the microbiota in regulation of IDO [22].

T cells can also play an important role in intestinal immunity. Commensal bacteria mediate intestinal immune tolerance by producing short-chain fatty acids, including acetate, propionate, and butyrate, through the fermentation of undigested carbohydrates. These have been shown to induce colonic Tregs through up-regulation of gut-homing molecules [23] and Foxp3 [24]. A subset of bacteria from the order Clostridiales has been identified as important for induction of colonic Tregs [25,26], potentially by up-regulating transforming growth factor-β to support Foxp3 induction. In contrast, pathogen-associated stimuli cause inflammatory responses via IL-1 and IL-6 induction, resulting in subsequent Th1 and Th17 activation [27].

B cells are an additional arm of the immune system active within intestinal tissues. Commensals have recently been shown to regulate B cell development within intestinal lamina propria [28]. IEC secretion of cytokines induces B cell class switching to IgA in a T cell–independent and T cell–dependent manner. IgA produced by local plasma cells is transported by IECs across the epithelial barrier into the intestinal lumen to act as another important line of defense against microbes [8].

In addition to myeloid cells and B and T lymphocytes, the recently described innate lymphoid cells (ILCs) [29,30] are also important in intestinal immune homeostasis. ILCs are classified into 3 distinct populations (groups 1, 2, and 3) on the basis of the expression of specific transcription factors, cell surface markers, and ability to secrete particular cytokines. RORγt-expressing group 3 ILCs in the intestine have