Review

Shuttling of information between the mucosal and luminal environment drives intestinal homeostasis

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ABSTRACT

The gastrointestinal tract is a passageway for dietary nutrients, microorganisms and xenobiotics. The gut is home to diverse bacterial communities forming the microbiota. While bacteria and their metabolites maintain gut homeostasis, the host uses innate and adaptive immune mechanisms to cope with the microbiota and luminal environment. In recent years, multiple bi-directional instructive mechanisms between microbiota, luminal content and mucosal immune systems have been uncovered. Indeed, epithelial and immune cell-derived mucosal signals shape microbiota composition, while microbiota and their by-products shape the mucosal immune system. Genetic and environmental perturbations alter gut mucosal responses which impact on microbial ecology structures. On the other hand, changes in microbiota alter intestinal mucosal responses. In this review, we discuss how intestinal epithelial Paneth and goblet cells interact with the microbiota, how environmental and genetic disorders are sensed by endoplasmic reticulum stress and autophagy responses, how specific bacteria, bacterial- and diet-derived products determine the function and activation of the mucosal immune system. We will also discuss the critical role of HDAC activity as a regulator of immune and epithelial cell homeostatic responses.

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1. Introduction

The gastrointestinal tract is a passageway for environment-derived products including dietary nutrients, external antigens, microorganisms and xenobiotics. The gut is home to diverse and abundant bacterial communities forming the microbiota. Indigestible-nutrient metabolism by resident enzyme-producing bacteria supply the host with numerous metabolites required for intestinal epithelial and immunologic maturation. Indeed, germ-free (GF) mice display maturation defects of both mucosal epithelial and lymphoid-associated development. In addition, bacteria and their by-products protect against pathogen invasion and maintain gut homeostasis. Likewise, the host uses a number of innate and adaptive immune mechanisms to cope with the intestinal microbiota and luminal environment, and to safeguard host-microbiota mutualism [1]. Both innate and adaptive immune cells populate the intestinal mucosal immune system. Innate immune cells include intestinal epithelial cells (IEC), neutrophils, dendritic cells, macrophages and innate lymphoid cells (ILCs). Intestinal mucosal adaptive cells include T-regulatory cells (T-reg) and Th17 cells. By

secreting anti-inflammatory cytokines such as IL-10 and TGF-β, T-reg cells suppress abnormal immune responses and are thus important for gut tolerance. Th17 cells, while crucial to host defense against infection, may become pathogenic as a result of aberrant regulatory mechanisms, as those observed during intestinal inflammation. In recent years, multiple bi-directional instructive mechanisms between microbiota, luminal content as well as mucosal innate and adaptive immune systems have been uncovered. Indeed, epithelial or immune cell-derived mucosal signals shape microbiota composition, while the microbiota and its by-products shape the mucosal immune system. Genetic as well as environmental perturbations lead to defects in gut mucosal cell responses which impact on microbial ecology structures, causing dysbiosis. On the other hand, changes in microbiota alter intestinal mucosal cell responses. In this review, we will discuss selected examples showing (1) how the intestinal epithelium forms the microbiome and controls gut microbial ecology, (2) how environmental and genetic perturbations are sensed through endoplasmic reticulum (ER) stress and autophagy responses in IECs, (3) how homeostatic responses are challenged in response to stress, and (4) how specific bacteria, bacterial- and diet-derived products determine the function and activation of the mucosal immune system. We will also discuss the intriguing possibility that regulation

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of histone deacetylase (HDAC) activity may be critical – not only for establishing immune-suppressive responses through T-reg cell activation, but also for controlling IEC homeostatic responses to the luminal environment.

2. The intestinal epithelium serves as a physical and biochemical protective barrier

The mono-layered intestinal epithelium is central to intestinal homeostasis – not only by way of its position between luminal microbiota and the mucosal immune system, but also by its capacity to sense and to respond to their respective signals (Fig. 1) [2,3]. Continuous IEC renewal is sustained by crypt stem cells whose differentiation and maintenance are regulated by different pathways [4]. Crypt stem cells generate multiple IEC lineages with distinct functions, including absorptive enterocytes, mucin-producing goblet cells, enteroendocrine cells and antimicrobial peptide (AMP)-secreting Paneth cells. The intestinal epithelium senses bacterial products through innate immune receptors such as Toll-like receptors and Nod-like receptors. This leads to activation of signalling pathways regulating IEC migration and tissue repair, among others [3]. The intestinal epithelium forms a protective physical barrier by establishing a web of tight junctions regulating intestinal permeability. Barrier function is defective in Inflammatory Bowel Disease (IBD) patients, and experimental colitis models involve innate response defects [5]. For example, TLR5-deficient mice display spontaneous intestinal inflammation that may be caused by a decreased ability to clear bacteria [6].

The intestinal epithelium also provides a chemical protective barrier which keeps microbes at bay and influences microbiota distribution and content. Two secretory IEC lineages, namely goblet and Paneth cells, are critical for this function. Goblet cells form a physical and chemical defense barrier by producing transmembrane mucin glycoproteins and by secreting mucins – notably Muc2 – covering the intestinal epithelium and forming a two-tiered inner and outer layer to prevent bacterial adhesion to the epithelium (Fig. 1). Goblet cells also express AMPs sequestered in the mucinous gel [7,8]. While the inner dense mucous layer restricts bacterial penetration and growth, the extended outer layer forms a well-suited environment for resident bacteria [9]. The importance of mucous protection in gut homeostasis is demonstrated by the development of spontaneous colitis in Muc2-deficient mice [10], and by reduced goblet cell numbers and depleted mucous secretion in IBD patients [11]. In addition, glycosylated mucin proteins are metabolized by specialized mucous-degrading enzyme-producing bacteria. Released oligosaccharides are used as a food source for growth of specific bacterial subsets – notably Bacteroides fragilis and Akkermansia muciniphila, among others. Thus, host mucin levels could impact the abundance and distribution of defined intestinal bacterial subsets [12].

A novel role for Muc2 as a direct signalling intermediate, controlling dendritic cell (DC) immunoregulation and small intestinal tolerance, has recently been uncovered [13]. Intestinal mucosal DCs display luminal bacterial antigens to gut immune cells [14]. LPS-induced DCs treated with glycosylated Muc2 show reduced expression of inflammatory cytokines. In contrast, Muc2 treatment induces the expression of anti-inflammatory and tolerogenic cytokines IL-10 and TGFβ, as well as retinaldehyde dehydrogenase ALDH1A1, which converts vitamin A into the T-reg inducer retinoic acid. Muc2-deficient IECs express decreased IL-10 and ALDH1A1 levels independently of the presence of bacteria. Gavage of Muc2 in Muc2-deficient mice results in increased expression of IL-10 and ALDH1A1, among others, correlating with increased T-reg cell numbers as well as decreased Th17 cell numbers. Interestingly, Muc2 administration reduces dextran sulphate sodium (DSS)-induced colitis symptoms in Muc2-deficient mice. Glycosylated

Muc2-dependent signalling on DCs involves the formation of a galectin 3-Dectin1-FcγRIIB receptor whose activation leads to β-catenin-dependent inhibition of inflammatory NF-κB inflammatory signals. Thus, in addition to its role as a protective epithelial barrier from bacterial adhesion and luminal antigens, and as a food source for specific bacteria, Muc2 may serve as a signalling molecule directly favouring DC tolerogenic – as opposed to inflammatory – responses, or indirectly through IEC-specific modulation of DC regulators (Fig. 1).

The small intestine's secretory Paneth cells are juxtaposed to intestinal epithelial stem cells in the crypt. The former are long-lived cells that secrete stem cell-supporting factors, establishing the stem cell niche [15], and transmit diet-related changes – such as caloric restriction – to modulate stem cell function [16]. Paneth cells secrete a trove of AMPs, including α-defensins and Reg lectins, which protect against pathogens and regulate microbiota composition (Fig. 1) [17,18]. Indeed, constitutively expressed defensins regulate global bacterial communities [19]. For example, expression of the human defensin DEFA5 gene in murine Paneth cells leads to changes in microbiota composition – notably reduction of segmented filamentous bacteria (SFB) – without affecting microbial loads. This results in mucosal immune cell modifications, including a decrease in IL-17-expressing T cells [19]. The Reg3g antibacterial C-type lectin – expressed by most IEC lineages – kills Gram-positive bacteria by binding bacterial peptidoglycan and forming an oligomeric membrane-invading pore [20,21]. Reg3g expression depends on IEC Myd88-TLR signalling, as mice deficient in Myd88 – a common adapter in TLR signalling pathways – display reduced Reg3g expression and increased bacterial interaction with the epithelium. Reg3g-deficient mice show increased bacteria-epithelium association and basal mucosal inflammatory responses. Thus, Reg3g is considered a regulator of surface-associated bacterial content [22,23]. Recent data have suggested an interaction between intestinal intraepithelial lymphocytes (IELs) and Paneth cells. IELs are specialized T immune cells in close contact with mucosal IECs. In response to bacteria and regulatory immune cells, IELs produce numerous cytokines which regulate mucosal barrier properties, among others [24]. While mice lacking intestinal IELs show reduced levels of Ang4 – an AMP produced by Paneth cells – the transfer of wild-type IELs to such mutant mice restores Ang4 expression. This IEL–IEC bi-directional interaction may involve signalling first from IECs to IELs, through IEC IL-23-dependent stimulation of IELs, and second from IELs to IECs, through IEL IL-22-specific activation of Ang4 in Paneth cells. This IEL–IEC interaction could be an important regulator of intestinal homeostasis in the wake of bacterial challenges [25].

3. ER stress and autophagy are internal intestinal epithelial cell sensors of environmental perturbations

The intestinal epithelium is constantly exposed to luminal substances including microbes and diet – as well as bacterial-derived products. Paneth and goblet cells, by their extensive synthesis of secretory products – requiring high metabolic energy levels – and by their exquisite protein folding capabilities, are very sensitive to environmental changes and stresses. Genome-wide association studies have identified genetic variants with enhanced risk of IBD, such as Crohn's disease (CD) and ulcerative colitis (UC). Of the numerous pathways identified, three major interacting pathways, namely innate signalling through NOD proteins, autophagy and ER stress responses, are integral to disturbed homeostatic intestinal responses associated with Paneth and goblet cell defects (Fig. 2) [26,27]. Nod2 is a cytosolic innate Nod-like receptor that binds bacterial muramyl dipeptide, leading to the activation of the pro-inflammatory transcription factor NF-κB [28]. Nod2 is a susceptibility locus for CD, an IBD disease associated with defective