Feature Review

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Focus: Immunity and the microbiota

Modulation of immune development and function by intestinal microbiota

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The immune system must constantly monitor the gastrointestinal tract for the presence of pathogens while tolerating trillions of commensal microbiota. It is clear that intestinal microbiota actively modulate the immune system to maintain a mutually beneficial relation, but the mechanisms that maintain homeostasis are not fully understood. Recent advances have begun to shed light on the cellular and molecular factors involved, revealing that a range of microbiota derivatives can influence host immune functions by targeting various cell types, including intestinal epithelial cells, mononuclear phagocytes, innate lymphoid cells, and B and T lymphocytes. Here, we review these findings, highlighting open questions and important challenges to overcome in translating this knowledge into new therapies for intestinal and systemic immune disorders.

The intestinal microbiota contributes to health and disease in the gut, and beyond

Advances in 16S ribosomal RNA sequencing have revealed the intestinal microbiota to be an incredibly complex community, comprising thousands of bacterial species in humans that vary markedly in distinct regions of the intestinal tract [1,2]. Although every human harbours a unique microbiota, there is a common pattern at the phyla level, with Bacteroidetes and Firmicutes (dominated by Clostridia) being the most abundant [2]. Novel metagenomic approaches have further clarified the composition of the microbiota, which will be useful in identifying the potential roles of distinct bacteria in intestinal homeostasis [3,4].

Fundamental roles of the microbiota in mammalian physiology have been derived from studies of germ-free (GF) or antibiotic-treated animals, demonstrating that the microbiota aids in food digestion, nutrient supply, and resistance to pathogenic infection [2]. Furthermore, GF animals exhibit impaired immune development, characterised by immature gut-associated lymphoid tissues (GALT), decreased numbers of intestinal lymphocytes, and diminished levels of antimicrobial peptides and immunoglobulin (Ig) A, all of which are reversed upon colonisation with commensal bacteria [5]. Additionally, maturation of the immune system appears dependent on host-specific commensals, because it did not occur in GF mice colonised with human microbiota [6], although this was recently challenged by the finding that selected species of human microbiota induced a population of regulatory T cells (Tregs) in the intestine of GF mice [7,8]. Despite promoting GALT development, not all members of the intestinal microbiota are beneficial; some may act as opportunistic pathogens, overabundance of certain commensals may predispose to pathogenic infection [9], and antibiotic-mediated alterations in commensal microbiota can predispose to nosocomial infections [10]. Interactions with intestinal microbiota may even facilitate infection by other enteric pathogens, as has been demonstrated for certain viruses and parasites (Box 1).

Furthermore, accumulating evidence suggests a correlation between inflammatory bowel disease (IBD), encompassing ulcerative colitis and Crohn's disease, and altered microbiota, a state termed 'dysbiosis', although whether dysbiosis is a primary cause of IBD or arises as a consequence of chronic intestinal inflammation remains unclear [11]. Nevertheless, in individuals with predisposing genetic or environmental abnormalities, intestinal microbiota are the focus of the aberrant host immune responses that drive the chronic inflammation characteristic of IBD [11]. An important caveat of many experimental studies linking dysbiosis to disease susceptibility in particular genotypes is the demonstration that familial transmission of microbiota from mother to neonate can have a dominant role in conferring distinct microbiotas (Box 2).

More recently, it has become clear that the influence of the microbiota extends beyond the intestinal tract and affects the systemic immune system. For instance, GF mice exhibited resistance to experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), but disease susceptibility was restored upon colonisation with microbiota, or by monocolonisation with segmented filamentous bacteria (SFB) [12–14]. Another interesting finding linked intestinal microbiota to gender-dependent susceptibility to type 1 diabetes mellitus (T1DM) in the nonobese diabetic (NOD) mouse model [15]. Female NOD mice are more susceptible to T1DM than are males, a phenotype that is equalised under GF settings. Moreover, transfer of caecal contents from male into female NOD mice corrected the susceptibility in a manner that was dependent on androgen receptor signalling and correlated with increases in testosterone levels

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Box 1. Exploitation of intestinal microbiota by enteric viruses and helminths

Intestinal microbiota affect the ability of the host to deal with other classes of pathogen, given that GF mice show enhanced susceptibility to many infections [130]. More recent studies have provided insight into some of the mechanisms involved. Antibiotic treatment to deplete intestinal microbiota revealed that tonic stimulation of innate immune circuits by the microbiota enhanced protective adaptive immune responses to influenza virus infection in the respiratory tract and to systemic infection with lymphocytic choriomeningitis virus [131,132]. Conversely, other studies show that some enteric viruses exploit the commensal microbiota to promote infection. The retrovirus, mouse mammary tumor virus (MMTV), is transmitted to neonatal mice in the milk of infected mothers; however, ablation of the intestinal microbiota prevented MMTV transmission to the offspring [133]. Furthermore, MMTV bound microbiota-derived LPS forming a complex that triggered TLR4-dependent IL-10 secretion by myeloid cells, engendering tolerance towards the virus to facilitate transmission [133]. Similarly, antibiotic-mediated depletion of microbiota induced resistance to enteric infection with reovirus and poliovirus [134]. Mechanistically, binding of bacterial LPS enhances poliovirus infectivity by increasing virion stability and by enhancing binding to the poliovirus receptor [135]. Furthermore, the reactivation of pathogenic endogenous retroviruses observed in antibody-deficient mice was dependent on the presence of the microbiota [136]. These studies reveal new aspects of the influence of the microbiota on viral infections and suggest that the use of antibiotics and probiotics during viral infections should be carefully considered.

Recently, Iliev *et al.* performed high-throughput sequencing to define the mouse intestinal fungal 'mycobiome', containing over 200 fungal species [137]. Furthermore, they found that mice deficient in Dectin-1, a key PRR that senses fungal β-glucans, exhibited greater susceptibility to DSS-induced colitis that was attenuated by treatment with an antifungal drug [137]. Finally, they identified a single nucleotide polymorphism (SNP) in Dectin-1 (*CLEC7A*) that was associated with more severe ulcerative colitis in humans [137]. Thus, impaired immunity to commensal fungi may exacerbate pathological inflammatory responses in the gut, but the mechanisms involved remain to be identified.

A key component of the intestinal fauna with which mammals have coevolved are multicellular parasites, especially helminth worms that frequently colonise the gastrointestinal tract [138]. Helminth infections, or treatment with the immune suppressive factors that they produce, can inhibit various immunopathological disorders, including models of IBD [138]. Intestinal helminths may indirectly regulate immune responses against intestinal microbiota through 'bystander' immune suppression. In addition, mice infected with the parasitic nematode *Heligmosomoides polygyrus* had altered ileal microbiota composition [139]. Conversely, commensal bacteria may in some cases have a critical role in facilitating infection by metazoan parasites. Contact with intestinal microbiota was shown to promote hatching of eggs of the mouse intestinal nematode *Trichuris muris* and antibiotic-mediated depletion of the microbiota resulted in reduced worm burdens [140]. Thus, interactions with intestinal microbiota appear to trigger the parasite to hatch in the appropriate niche and type I fimbriae were shown to be capable of mediating this interaction [140].

Finally, in addition to interactions with other classes of pathogen, much remains to be discovered regarding how interspecies interactions amongst the bacterial microbiota may impact on intestinal homeostasis (see the review by Kaiko and Stappenbeck in this issue [141]). Therefore, within the complex environment of the gut, both host–microbe and microbe–microbe interactions can profoundly influence local and systemic immune homeostasis.

[15]. Further implicating a role for the microbiota in metabolic disease, shifts in microbiota composition were recently associated with the progression of the liver disorder nonalcoholic steatohepatitis (NASH) [16]. Thus, mice deficient in the inflammasome-associated, cytosolic innate immune receptors NOD-like family, pyrin domain containing (NLRP) 3 or NLRP6, displayed exacerbated NASH that was due to a dysbiotic microbiota, because wild type mice cohoused with the inflammasome-deficient mice also showed increased susceptibility to NASH [16]. Additional examples of the influence of intestinal microbiota on autoimmune disorders include studies in mouse models of inflammatory arthritis and autoimmune polyglandular syndrome [17].

Box 2. A cautionary note on microbiota associations with genotype and disease susceptibility

Although a plethora of studies have implicated microbiota dysbiosis with increased disease phenotypes in transgenic mice [142], such as those deficient in PRR signalling, a key study highlighted the risk of potential false positive associations in these types of investigation. Ubeda *et al.* showed that *Myd88*$^{-/-}$ and *Tlr*$^{-/-}$ mice exhibited distinct microbiotas from each other, but that these were primarily shaped by maternal transmission rather than host genotype [143]. Thus, by crossing heterozygous *Myd88*$^{+/-}$ mice or *Tlr*$^{+/-}$ mice to generate wild type and knockout mice from the same litter, they demonstrated that there was little difference in the intestinal microbiota composition of wild type and TLR-deficient littermates [143]. Indeed, the microbiota of wild type littermates more closely resembled that found in their *Myd88*$^{-/-}$ littermates than that present in wild type mice from other colonies [143]. Thus, some of the reported incidences of 'dysbiosis' between wild type and knockout mouse strains may in fact reflect divergence of microbiota that occurs during long-term breeding of isolated colonies, which is perpetuated by maternal transmission. This elegant study shows that littermate controls are crucial in differentiating between dysbiosis that may emerge stochastically and dysbiosis that is causally related to the genotype of the host and, therefore, should be used in future studies in this area.

Together, these studies highlight that the microbiota have a wide range of effects on the development and responsiveness of the local and systemic immune system, but the mechanisms responsible are incompletely understood. Here, we review recent progress that has begun to elucidate some of the cellular and molecular factors involved. We outline how structural moieties and metabolites derived from the intestinal microbiota act on intestinal epithelial cells (IEC) and local innate leukocytes to maintain barrier defence and regulate immune homeostasis. We then describe how microbiota-derived factors activate a multitude of pathways that control adaptive immunity in the gut, by promoting IgA secretion and regulating the balance between effector and regulatory T cells. Finally, we note some of the key issues that remain to be addressed to translate this improved understanding into novel treatments for infections and inflammatory diseases.

Sensing of microbiota by IECs maintains intestinal homeostasis

IEC form a single cell barrier layer on the surface of the intestinal mucosa and, although not considered *bona fide* immune cells, their interactions with intestinal microbiota influence the immune response and have a crucial role in maintaining homeostasis [18].