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Perspective

Commensals, Bacterial Pathogens and Intestinal Inflammation: An Intriguing Ménage à Trois

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According to a classical view of bacterial-host interactions at intestinal surfaces, the commensal microbiota establishes tolerance, and invasive pathogens cause stereotypic inflammation. The reality is more complex, marked by a "ménage à trois" situation encompassing three emerging concepts: (1) pathogens take advantage of inflammation to cross the epithelial barrier, (2) pathogens reduce the commensal flora to invade their niche, and (3) pathogens express dedicated effectors that modulate inflammation.

Introduction

Intestinal inflammation occurs when an enteric pathogen bypasses barriers imposed by the commensal gut flora, and the epithelial lining, or when innate immune defects that largely remain to be identified, disrupt tolerance to the resident microbiota. The later results in inflammatory bowel diseases (IBDs), Crohn's disease being a typical example. Here, we review and discuss the tumultuous relationship entertained among bacterial pathogens, commensals, and inflammation, a "ménage à trois" that is emerging as a new paradigm in pathogenesis. Intestinal inflammation, the stereotypical host innate response to a bacterial pathogen, is expected to facilitate the pathogen's eradication. In reality, a much more subtle game is engaged in which the pathogen subverts inflammation to escape its lethal effect, and concomitantly can take advantage of inflammation to breach the barrier effect imposed by the resident flora and the epithelium itself. We will outline the dynamic mechanisms by which the resident microbiota and the mucosa conjugate efforts to maintain homeostasis, and how it is disrupted by pathogens. We will also consider how pathogens use the inflammation they elicit to subvert the integrated barrier established by the resident flora and the epithelium, and how they eventually suppress this inflammation in order to secure their colonization and full invasion potential.

The Commensal, the Pathogen and the Epithelium: A Ménage à Trois

Commensal bacteria that colonize the gut protect the host from intruding pathogens by imposing a colonization barrier, also called the barrier effect (Stecher and Hardt, 2008). This protective community composed of 500–1000 species of microorganisms reaching a concentration of 10^{11} bacteria per gram of colon content cannot be ignored by the host. Commensals as well as pathogens are characterized by the presence of molecular patterns (PAMPs) that are specific to the prokaryotic world. As a complement, the epithelial lining and associated cells express an array of pathogen-recognizing receptors (PRRs) that, upon activation by the PAMPs, should induce inflammation (for details, see the Review by Ishii et al., page 352 in this issue). Clearly this is not always the case, as chronic inflammation is detrimental to the host. Thus, host defenses are able to accurately interpret the microbial environment in order to discriminate between permanently established commensal microbes and episodic pathogens.

One can envision four major sets of parameters supporting the largely active tolerogenic process that maintains homeostasis. (1) Bacteria may escape or alter the inflammatory response. They may remain "stealth," due to yet-to-be explored diversity in PAMPs, such as pentacylated lipidA of anaerobic gram-negative commensals (i.e., *Bacteroidetes*) that are unable to signal via TLR4, the poor agonist activity of flagellin for TLR5, or variability in peptidoglycan. Commensal bacteria may also actively suppress epithelial proinflammatory signaling, as demonstrated for *Bacteroides thetaiotaomicron* that induce export of RelA, the p65 subunit of the proinflammatory transcription factor NF-κB, out of the nucleus leading to decreased transcription of NF-κB-dependent genes (Kelly et al., 2004), and for *Lactobacillus casei* that suppress degradation of the inhibitor of NF-κB, I-κB (Tien et al., 2006). (2) The host may express factors and enzymes that assist in tolerating the commensal by blunting microbial components that would typically induce inflammation. For example, recent evidence indicates that the brush border alkaline phosphatase expressed in the intestinal epithelium can detoxify luminal LPS by dephosphorylating the lipid A (Bates et al., 2007). (3) There is also a physical dimension to the tolerogenic process. This includes the combined production of mucus and antibacterial molecules, particularly antimicrobial peptides (AMP), by the epithelial lining (Liévin-Le Moal and Servin, 2006). The mucus serves as a matrix for the AMPs secreted by epithelial cells, and together they are likely to maintain commensals restrained and separated from the epithelial surface. Consistent with this hypothesis of a "no-bacteria zone" created over a certain distance from the epithelial surface is the observation that in Crohn's disease,

which is marked by strong decrease in expression of AMPs by the intestinal and colonic epithelium (Nuding et al., 2007), bacteria gain close access to the epithelial surface on which they grow as a thick layer (Swidsinski et al., 2002). Further, in such a situation the diversity of the microbiota gets reduced (Manichanh et al., 2006), indicating a strong impact of chronic inflammation on the composition of the resident flora. (4) The third component is a more immunological one. A significant sequestration of PRRs seems to exist with little presence of TLR4 and/or coactivation molecules (i.e., MD2 and CD14) in the gut surface epithelium, thus a certain degree of "blindness" of the surface most exposed to the resident flora (Abreu et al., 2005). Also, the epithelial lining gets tolerized to LPS very early in life (Lotz et al., 2006), and the integrated mucosal immune system is strongly oriented to tolerance, with epithelial signals such as the production of Thymic stromal lymphopoietin orienting T cell responses toward noninflammatory (i.e., non Th1) responses (Rimoldi et al., 2005). T regulatory lymphocytes (Treg) producing IL-10 and TGF-β are essential final effectors of tolerance to the resident flora (Izcue et al., 2006). Last but not least, the adaptive immune system is enrolled in the tolerogenic process through the local production of commensal-specific IgA(s) that seem, experimentally, to be able to reduce intestinal proinflammatory signaling (Peterson et al., 2007; also see Review by Peterson et al., page 417 in this issue).

It is clear that pathogens have the capacity to subvert the four levels of security defined above by managing close access to the epithelial surface, then defying the mucosal innate immune network of microbial sensing by delivering PAMPs in close proximity to epithelial sensors, and ultimately by invading the tissue. Altogether, these signals incite a rapid inflammatory host response characterized by secretion of proinflammatory cytokines and chemokines attracting neutrophils, monoytes, and DCs to the site of infection aimed at bacterial eradication. This phase corresponds to the first level of complexity in the ménage a trois.

Diversion of Inflammation by Pathogens

Pathogens exploit host inflammation to colonize and/or invade their host. Although this may seem like an emerging theme, it was shown more than 40 years ago that *Listeria monocytogenes* invades recruited monocytes and uses them as vehicles to spread to distant tissues (Gray and Killinger, 1966). In the case of *Shigella* infection, blocking the recruitment and epithelial transmigration of neutrophils by systemic administration of an anti-CD18 monoclonal antibody prevented the rupture of the epithelial barrier's coherence and thereby blocked both inflammation and bacterial invasion of the epithelial lining (Perdomo et al., 1994). This experiment illustrated the capacity of an inflammatory infiltrate to facilitate the passage of a host barrier. From the perspective of the pathogen, this access was gained at the cost of bacterial killing and thus at the risk of abortive infection. However, the central question is whether this somewhat provocative concept can be generalized to other chronic and acute infection systems and whether inflammation, through rupture of the epithelial barrier, is a primary contributor to bacterial infection.

It has been shown that proinflammatory cytokines, such as IFN-γ and TNF-α, can disrupt the epithelial barrier by inducing increased paracellular permeability via tight junctions disruption (Bruewer et al., 2003). Moreover, gastric epithelial inflammation seems to be vital for *Helicobacter pylori* to establish long-term colonization (Mimuro et al., 2007). Inflammation can also be a secondary contributor by eliminating a significant part of the resident flora, thereby altering its colonization barrier effect. Studies indicating that antibiotic treatment facilitates gut invasion by enteric bacteria support the idea that changes in the intestinal flora allow infection by pathogens (Beaugerie and Petit, 2004). Emerging evidence suggests that pathogens can themselves alter the resident flora, a grand classic in ménage à trois, where the lover kills the husband with his mistress' complicity. This was recently described in a mouse model of gut infection by *Citrobacter rodentium* and *Campylobacter jejuni*, which are murine enteric pathogens similar to enteropathogenic *E. coli*. Comparing with chemically and genetically induced models of gut mucosal inflammation, the authors showed that as bacterial infection proceeds, the resident colonic microflora undergo reduction and simplification, whereas the potentially pathogenic aerobic bacteria, particularly Enterobacteriaceae, flourishes. These drastic changes of the resident flora clearly correlated with facilitation of *C. rodentium* infection (Lupp et al., 2007). Although the mechanisms promoting these changes remain unclear, one can hypothesize that bactericidal mediators produced by the inflamed epithelium in response to bacterial infection (e.g., reactive oxygen radicals, NO, AMPs) result in microbial selection based on the relative intrinsic resistance of the resident and invading species to these effectors. This is somewhat similar to the situation with Crohn's disease, during the course of which a subpopulation of resistant species that can live in close contact with the epithelial surface is selected (Conte et al., 2006). The selected bacterial species include Enterobacteriacea, and possibly particularly resistant bacteria such as the new class of adherent-invasive *E.coli* (Barnich et al., 2007) that are currently considered hypothetical disease-causative agents. Mice infected with *Salmonella Enterica* serovar *Typhimurium* also exhibited reduction and simplification of the resident microflora, for example a decrease in *Lactobacillus* and *Bacteroides spp*., these changes being correlated with expression of *S. Enterica* virulence factors (Stecher et al., 2007; Barman et al., 2008). It has been suggested that the inflammatory mucosa represents a source of nutrients that *Salmonella* use for their growth (Stecher and Hardt, 2008). From a Darwinian perspective, mucosal inflammation that has been "mastered" by pathogens may provide these bacteria with variety of options, such as solving particular metabolic needs, eliminating the colonization barrier of the commensal flora, and disrupting the physical barrier of the epithelium. Such a hypothesis holds true only if pathogens can actually "master" inflammation; otherwise, they are likely to incinerate in the fire they lit.

Subversion of Inflammation by Pathogens

Among their capacities to manipulate a broad array of pathways in their target cells, enteropathogenic bacteria have developed sophisticated strategies to master inflammation (Bhavsar et al., 2007). They alter inflammation qualitatively and quantitatively in a way compatible with

bacterial survival and preservation of growth capacities, to enhance colonization potential and, if required, invasion. Bacterial effectors are now recognized to directly modulate proinflammatory pathways in order to limit detrimental inflammation, although in vivo confirmation is still often warranted. A *Shigella* effector, OspG, inhibits the NF-κB pathway. It is a kinase that binds a subset of ubiquitin-conjugating enzymes (E2s), thereby preventing ubiquitination of phosphorylated I-κB, which is consequently not degraded by the proteasome, making OspG a potent anti-inflammatory effector in an in vivo model of infection (Kim et al., 2005). Yet another *Shigella* effector, OspF, is a phosphothreonine-lyase that dephosphorylates two MAPKs (p38 MAPK and ERK2) in the nucleus, blocking the phosphorylation of histone H3 on Ser^{10}, and consequently inhibiting activation of genes under NF-κB control. This mechanism translates in vivo to the regulation of recruitment and transmigration of neutrophils to the site of infection (Arbibe et al., 2007). Epigenetic regulation of inflammatory responses by pathogens may turn out to represent a major strategy able to regulate restricted sets of proinflammatory cytokines, and possibly imprint this regulation for long periods in the course of the infectious process. Haller and colleagues showed that TGF-β1 expression induced by *Bacteroides vulgatus* inhibits NF-κB activation through histone acetylation (Haller et al., 2003). Listeriolysin O, a membranolytic toxin secreted by *L. monocytogenes*, was shown to modulate host gene expression by inducing dephosphorylation of Ser^{10} on histone H3, and deacetylation of histone H4, leading to decreased expression of proinflammatory chemokines like CXCL2 (Hamon et al., 2007). In addition to OspF regulating neutrophil infiltration, Osp and IpaH effectors, a novel family of E3 ligases (Rohde et al., 2007), were recently shown to collectively suppress expression of AMPs, particularly human β-defensin-3 (HBD3) and cathelicidin LL-37, that are bactericidal to *Shigella* (Spérandio et al., 2008). "Spilling oil on the fire" cannot be a sustainable strategy for a pathogen, which will eventually perish in this risky strategy. It is thus not surprising that, under such strong selective pressure, enteropathogens have accumulated a collection of dedicated regulators of the host innate response. Compromising is a general rule, even in ménages à trois.

Conclusion

Future studies will make increasing sense of the molecular strategies used by pathogens to "carve" a host innate response that is compatible with their survival and proliferation. It is already clear that in niches that harbor a permanently resident flora, pathogenesis cannot be considered under the simplistic angle of a dual host-pathogen interaction. It is a ménage à trois, a three-partners' story that promises much more exciting scenarios.

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