Published in final edited form as:

Nat Med. ; 18(5): 654–656. doi:10.1038/nm.2767.

Germes gone wild

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Abstract

A normally peaceful behavior of numerous residents of the intestinal tract is maintained by community structure and the integrity of host defenses. When either or both of these are compromised, a few disgruntled outcasts can cause a riot taking down the whole neighborhood.

Trillions of bacteria colonize lower intestinal tract in mammalian species. The composition, diversity and stability of the intestinal microbial ecosystems have recently become a subject of intense investigation and a rich source of surprising findings. One notion that quickly emerged from these studies is that loss of microbial diversity at colonized tissues tends to be associated with deleterious effects on the host¹. Oral administration of antibiotics has one of the most powerful impacts on microbial composition and diversity and has long been known to disrupt the intestinal homeostasis, predisposing the host to infections with antibiotic-resistant pathogens². In this issue of *Nature Medicine*, Ayers *et al.*³ provides a detailed account of the lethal consequences of antibiotic-induced disturbance of the intestinal microbiota and the detrimental role of the innate immune system in decreasing tolerance in this setting.

Studies in germ-free animals have long established a crucial role of microbial colonization in the gut for normal development and physiology of the host organism⁴. Administration of broad spectrum antibiotics to adult animals bypasses the developmental effects of colonization and can recapitulate some of the phenotypes of germ-free animals, such as alteration of intestinal epithelial homeostasis and increased susceptibility to damage⁵. However, antibiotics do not completely sterilize the intestinal lumen and antibiotic-resistant bacterial strains can greatly expand in the newly available niches. The consequences of such an expansion depend on the host status—for example, barrier integrity and immune competence—as well as on the identity of antibiotic resistant bacteria.

The study by Ayers *et al.*³, showed how antibiotic treatment impacts on intestinal epithelial damage caused by dextran sodium sulphate (DSS). DSS is toxic to colonic epithelium and its oral administration is a convenient and widely used model of intestinal damage and colitis. Antibiotic-induced depletion of commensal bacteria was previously shown to increase susceptibility to DSS-induced intestinal injury⁵. In that model, commensal bacteria provided ligands for TLR stimulation, which, in turn, was necessary to promote epithelial tissue protection and repair. In the experimental model used by Ayers *et al.*³ antibiotic administration after DSS treatment resulted in severe mortality, but the cause of the problem seems to be a disruption of normal intestinal bacterial composition by antibiotics rather than lack of stimulation. Depletion of resident bacteria (symbionts) that normally dominate the intestinal niches resulted in the overgrowth of antibiotic-resistant strain of pathogenic *Escherichia coli* (*E. coli* O21:H+) (**Fig. 1**). Interestingly, the virulence gene cluster of *E. coli* O21:H+ has a characteristic feature shared with other septicemic *E. coli*.³ Indeed, invasion of *E. coli* O21:H+ into the systemic circulation caused sepsis-like condition with severe inflammatory tissue damage in multiple organs³. This invasion was facilitated by DSS-mediated disruption of intestinal barrier integrity. However, intravenous injection of the

bacteria into mice untreated with DSS was sufficient to cause sepsis and mortality, confirming the connection between this particular pathogen and the disease.

Interestingly, the lethal systemic inflammatory response triggered by *E. coli* O21:H+ was dependent on the production of interleukin-1 (IL-1 β) through the activation of NAIP5-NLRC4 inflammasome in macrophages. Inflammasomes are intracellular protein complexes that control proteolytic processing and secretion of IL-1 β and several other inflammatory mediators⁶. NAIP5-NLRC4 recognizes bacterial flagellin⁷, which can be secreted into the host cell through the specialized secretory apparatus known as ETT2 type 3 secretion system (T3SS)^{8,9}. *E. coli* O21:H+ isolated by Ayers *et al.*³ and related septicemic bacteria share a genetic variation (known as ETT2_{sepsis}) affecting their T3SS, suggesting that ETT2_{sepsis} may be particularly prone to flagellin secretion. It would be interesting to determine in future studies whether all bacteria sharing this particular version of the T3SS can cause sepsis through the flagellin–NLRC4–IL-1 β pathway. This would be important as it may inform the choice of therapy (such as IL-1 blockade) for sepsis patients, as sepsis can be caused by more than one mechanism, and in some settings TNF- α has a more dominant role compared to IL-1 β ¹⁰.

Immunopathology (collateral tissue damage caused by the immune response) is often an unavoidable cost of host defense from infections. The clinical challenge of dealing with immunopathology is that reducing the immune response can compromise host protection from an ongoing infection. How to strike the right balance between optimal host protection and minimal immunopathology is not always clear, and requires a detailed knowledge of the relative contribution of different host defense mechanisms to protection versus disease. In that context, another important finding by the authors is that the NLRC4–IL-1 β pathway does not seem to contribute to the clearance of the pathogenic *E. coli* O21:H+, as blocking this pathway did not affect pathogen burden, even though it did improve host survival³. This indicates that in this model of sepsis, interfering with the NAIP5–NLRC4–IL-1 β pathway might be beneficial without having negative consequences for host immunity. Furthermore, it can be therapeutic even in the face of high bacterial burden, which can be particularly relevant for antibiotic-resistant pathogens, such as *E. coli* O21:H+. Indeed, when infections progress to sepsis, the inflammatory response is a far greater evil than direct tissue damage by the pathogen—at that stage simply suppressing pathogen load alone has a limited therapeutic value. Therefore, identifying the pathways responsible for the inflammatory tissue damage—but dispensable for host immunity—as done by Ayers *et al.*³, is of utmost importance.

Finally, the study by Ayers *et al.*³ highlights another important emerging issue: the notion of pathobionts, microorganisms that can be part of normal microbiota in healthy hosts but that can cause disease when some aspect of host-microbial homeostasis is disrupted¹¹. The difference between pathobionts and opportunistic pathogens is that the latter are traditionally thought to be able to cause the disease when the host immunity is compromised. The definition of pathobionts can be considerably broader, as their pathogenic potential can be unleashed by multiple factors, particularly, alterations of microbial ecology in the colonized host¹². Perhaps the most famous microorganism exemplifying this principle is *Helicobacter Pylori*—although more than half of the human population harbors this bacterium in their stomachs, only a fraction develops ulcers¹³. The distinctions between beneficial commensal microbes, pathobionts and pathogens are not absolute, yet the emerging evidence suggests that in a given host, the presence and relative abundance of pathobionts seems to determine the susceptibility to multiple inflammatory diseases^{14,15}. The germ theory of disease proposed that many human maladies can be caused by specific pathogens. In the near future we may expect an expansion of the germ theory to incorporate the pathogenic potential of normal constituents of our microbiota.

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Figure 1. Epithelial integrity and microbial composition affects intestinal homeostasis and the innate immune response

(a) Pathobionts are normally present at low density in microbial community dominated by symbionts; (b) antibiotic mediated depletion of symbionts leads to expansion of antibiotic resistant pathobionts, however intact epithelial barrier prevents their invasion; (c) disruption of epithelial integrity can lead to a transient invasion of symbionts that are rapidly cleared by the immune system; (d) disruption of epithelial integrity in the context of antibiotic induced dysbiosis leads to invasion of pathobionts and subsequent septicemia. *E. coli* O21:H+ carries a variant of virulence gene cluster known as ETT2_{sepsis}, which may promote flagellin secretion through the type 3 secretion system (T3SS). Flagellin secreted into the host

macrophages activates the NAIP5–NLRC4–IL-1 β pathway ultimately leading to multiple organ damage caused by systemic effects of IL-1 β .