are likely the highly-glycosylated mucous proteins, which ovalbumin injected into the intestine more closely mimics.

On a more theoretical note, one might ask what purpose is served by the division of labor between macrophages and DCs—why can't the DCs take up the antigen, or conversely, the macrophages carry it to lymph nodes? Here Mazzini et al. speculate that this relay race might serve to prevent DCs from contacting the gut microbiota and becoming needlessly activated. Because the DCs have also been observed sending extensions into the lumen, and because transfer of antigens between myeloid cells has also been documented in other settings (Allan et al., 2006), this might not be the whole story. Regardless, Mazzini and colleagues have revealed that in the case of developing tolerance to ingested nutrients, food antigens must mind the gap.

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The Battle in the Gut

Denise M. Monack[1,*]
[1]Department of Microbiology and Immunology, Stanford School of Medicine, Stanford University, Stanford, CA 94305, USA
*Correspondence: dmonack@stanford.edu
http://dx.doi.org/10.1016/j.immuni.2014.01.007

Our molecular understanding of how pathogen-microbiota-immune system interactions influence disease outcomes is limited. In this issue of *Immunity*, Behnsen et al. (2014) report that the cytokine interleukin-22, which usually plays a protective role, promotes pathogen colonization by suppressing related commensal bacteria.

We are beginning to understand the complex interplay between mammalian immune systems, indigenous microbial communities and microbial pathogens at a molecular level. The human gut is teeming with trillions of bacteria that are essential for the maintenance of our health. For example, commensal bacteria are key participants in the digestion of food and extract nutrients and other metabolites that we need to stay healthy. Many of the metabolites and nutrients that commensal bacteria provide are implicated in the development, homeostasis and function of our immune system. Thus, our indigenous gut bacteria can provide protection to invading pathogens by influencing immune and nutritional barriers. In addition, commensal bacteria can provide increased resistance to bacterial pathogens by occupying their required niche. However, many bacterial pathogens have the capacity to disrupt or bypass homeostatic, immune, and colonization resistance mechanisms (Sansonetti, 2011). In this issue of *Immunity*, Behnsen et al. (2014) explore the complex interactions between an important mucosal immune factor, the commensal bacteria and the enteric pathogen *Salmonella enterica* serovar Typhimurium (referred as *Salmonella* from here on) in the guts of mice.

Salmonella is an important food-borne pathogen that causes a self-limited gastroenteritis in humans. The mucosal immune response to *Salmonella*, as with other pathogens, is orchestrated by T cells that express the cytokines interleukin-17 (IL-17) and IL-22. IL-17 promotes the recruitment of neutrophils and prevents the dissemination of *Salmonella* to the reticuloendothelial system. IL-22 is produced by immune cells, including T-helper cell subsets and innate lymphocytes, but acts only on non-hematopoietic stromal cells; in particular epithelial cells, keratinocytes, and hepatocytes (Rutz et al., 2013). IL-22 is usually beneficial to the host because it elicits the expression of proinflammatory epithelial defense mechanisms that are essential for host protection. IL-22 promotes epithelial proliferation and helps to maintain and restore the integrity of the epithelial barrier function during the invasion by pathogens. In addition, IL-22 synergizes with other cytokines, such as IL-17 or tumor necrosis factor-α (TNF-α), to induce expression of antimicrobial proteins involved in host defense in the skin, the airways, and the intestine. For example, IL-22 induces the expression of S100A7, S100A8, S100A9, β-defensin-2, and β-defensin-3 in the skin. It also promotes the release of RegIIIβ and RegIIIγ from intestinal cells and stimulates the production of protective mucus (Muc1, Muc3, Muc10, and Muc13) from

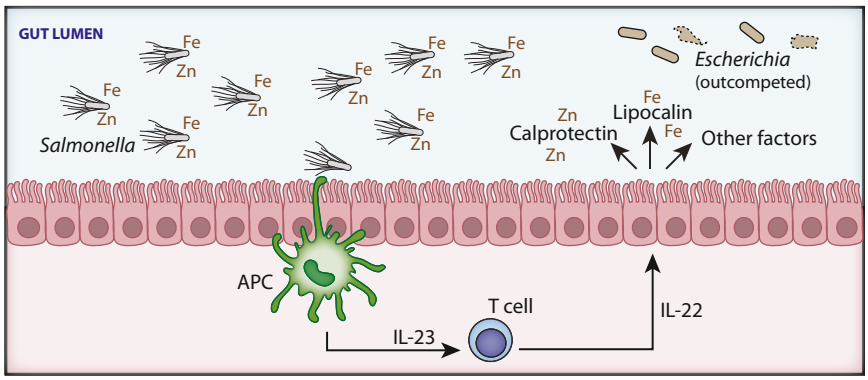

Figure 1. *Salmonella* Exploits IL-22-Mediated Nutritional Immune Mechanisms to Outcompete *Escherichia coli* in the Gut

In the gut, APCs are activated by *Salmonella* to produce IL-23, which engages T cells and other cell types to produce IL-22. IL-22 binds to receptors on colonocytes and promotes production of antimicrobial molecules including lipochalin and two subunits of calprotectin. Lipochalin and calprotectin bind metal ions, which are essential for bacterial replication. However, *Salmonella* expresses proteins (salmochelin and ZnuABC) that can steal metal ions from lipochalin and calprotectin and thus successfully outcompete its nearest neighbors, *E. coli* and other gut flora. In IL-22-deficient mice, there are fewer antimicrobial factors expressed and both *Salmonella* and *E. coli* colonize the gut.

goblet cells. Lastly, IL-22 promotes the production of inflammatory mediators, such as IL-6, G-CSF, and IL-1β, and plays a role in the release of chemokines, such as CXCL1, CXCL5, and CXCL9, from airway epithelial cells during infection (Rutz et al., 2013).

Salmonella thrives in the inflamed gut and successfully outcompetes the microbiota by mechanisms that are not completely understood (Winter et al., 2013). Behnsen et al. set out to elucidate the role of IL-22 during *Salmonella* infection in the gut of mice and quite surprisingly demonstrated that IL-22 does not play a protective role, but instead is exploited by this pathogen in order to cause gastroenteritis. Behnsen et al. orally infected streptomycin pretreated IL-22-deficient mice with *Salmonella* and found that the intestines of these mice contained significantly fewer *Salmonella* compared to the wild-type (WT) mice. Importantly, they could rescue *Salmonella* colonization by injecting infected *Il22*$^{-/-}$ mice with IL-22. This result was surprising because IL-22 has been shown to play a protective role in the guts of mice against *Citrobacter rodentium*, against vancomycin-resistant *Enterococcus* (Kinnebrew et al., 2010; Zheng et al., 2008), and against *Klebsiella pneumonia* in the lungs of mice (Aujla et al., 2008). Because previous studies suggested that *Salmonella* achieves high levels of colonization of the gut only when this organ is inflamed, the authors wondered whether the IL-22-deficient mice had less inflammation, which would explain the reduced *Salmonella* colonization. However, this was not the case and the differences in the levels of pathogen colonization cannot be explained by differences in the levels of inflammation.

Because *Salmonella* needs to compete with the microbiota in order to colonize the inflamed gut (Winter et al., 2013), the authors explored the possibility that the established microbiota is different in the absence of IL-22. When Behnsen et al. analyzed the gut microbiota compositions of uninfected WT and *Il22*$^{-/-}$ mice, they did not detect any significant differences. In contrast, Proteobacteria bloomed in the inflamed gut of both WT and *Il22*$^{-/-}$ mice infected with *Salmonella*. However, there was a considerable difference in the relative abundance of the genera *Salmonella* and *Escherichia* in the guts of these mice. While *Salmonella* constituted ~50% of the total bacteria in infected WT mice, it comprised 15% in the IL-22-deficient mice, whereas *Escherichia* constituted 40% of Proteobacteria. These results indicated that, in the absence of IL-22, commensal *Enterobacteriaceae* can compete with *Salmonella* in the inflamed gut.

What is the mechanism? IL-22 regulates antimicrobial responses (Rutz et al., 2013), such as the expression of lipocalin-2, which sequesters the siderophore enterochelin and inhibits growth of *Enterobacteriaceae*; S100a8 and S100a9, which are two subunits of calprotectin, an antimicrobial protein that sequesters zinc and manganese from pathogens; and enzymes that play a role in the generation of reactive nitrogen and oxygen species (iNOS and Duox2). The authors found that the expression of genes encoding metal binding proteins (Lcn2, S100a8, and S100a9) and those encoding proteins involved in the generation of reactive oxygen and reactive nitrogen species (Nos and Duox2) were significantly reduced in *Salmonella* -infected *Il22*$^{-/-}$ mice. Because *Salmonella* possess multiple virulence mechanisms that mediate resistance to specific antimicrobial proteins (Liu et al., 2012; Raffatellu et al., 2009; Stelter et al., 2011) and allow it to thrive in the inflamed gut (Winter et al., 2013), the authors speculated that *Salmonella* was surviving IL-22-dependent killing mechanisms to outcompete *Escherichia* in the guts of WT mice. However, in the absence of IL-22, the indigenous *Escherichia* could get the upper hand. To test this notion, Behnsen et al. assessed whether IL-22 enhances the colonization of *Salmonella* over isogenic mutant strains with known susceptibilities to IL-22-dependent antimicrobial proteins. To overcome calprotectin-mediated zinc sequestration by the host, *Salmonella* acquires iron with the siderophore salmochelin. Mutants in the salmochelin receptor are susceptible to iron sequestration by lipocalin-2 in the inflamed gut (Raffatellu et al., 2009). *Salmonella* overcomes another host defense mechanism mediated by calprotectin sequestration of zinc by transporting this essential metal via the high affinity ZnuABC system (Liu et al., 2012). To test the idea that these *Salmonella* virulence mechanisms are utilized to exploit IL-22-dependent immune mechanisms, the authors infected mice with mixtures of *Salmonella* mutants in these pathways (e.g., *iroN* and *znuA* deletion mutants) and show that they lose their growth advantage over commensal *E. coli*. When iron and zinc availability was limited by lipochalin-2 and calprotectin, iron acquisition through salmochelin and zinc acquisition through the ZnuABC transporter greatly enhanced the competitive advantage of *Salmonella* in the intestine of WT mice (Figure 1). However,

Salmonella loses its competitive advantage in the guts of *Il22*$^{-/-}$ mice where lipocalin-2 and calprotectin levels are reduced. Thus, *Salmonella* exploits IL-22, a key regulator of nutritional immunity, which starves microorganisms from essential metal nutrients, by expressing virulence factors that allow it to sequester these nutrients and outcompete commensal *Enterobacteriaceae*, its closest relative in the intestine.

In the future, it will be very important to determine whether IL-22, a key regulator of nutritional immunity, benefits other mucosal pathogens by similar mechanisms, i.e., by inducing antimicrobial responses that suppress the growth of the microbiota, thereby enhancing their colonization. It will also be important to identify additional IL-22-dependent antimicrobial factors. Finally, these findings suggest that specific targeting of virulence mechanisms that promote evasion of IL-22-mediated host defenses is a viable strategy to harness and control mucosal pathogens.

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Macrophage Activation: Glancing into Diversity

Gioacchino Natoli[1,*] and Silvia Monticelli[2,*]
[1]Department of Experimental Oncology, European Institute of Oncology (IEO), Milan 20141, Italy
[2]Institute for Research in Biomedicine (IRB), 6500 Bellinzona, Switzerland
*Correspondence: gioacchino.natoli@ieo.eu (G.N.), silvia.monticelli@irb.usi.ch (S.M.)
http://dx.doi.org/10.1016/j.immuni.2014.01.004

Macrophage activation is a crucial process for innate immunity as well as for tissue and metabolic homeostasis. In this issue of *Immunity*, Xue et al. (2014) extend our knowledge on macrophage activation and identify unique functional states, thus expanding the M1-M2 paradigm.

An essential requisite for macrophages to be able to exert their physiological functions is to accurately recognize and classify microenvironmental changes, in order to properly react to such challenges and also to coordinate both local and general responses. A critical component of this environmental response is often a broad transcriptional reprogramming involving hundreds of protein-coding and noncoding genes, a process whose final aim is the expression of gene products relevant to cope with possible emergencies (Smale, 2010). Although invading microorganisms represent the most relevant emergency that macrophages usually deal with, these cells also exert complex roles during development, tissue remodeling, and sterile damage repair (Wynn et al., 2013). Particularly in the case of systemic infections, the efficient removal of microorganisms often requires complex metabolic changes in the entire organism, explaining the extensive crosstalk between macrophages and cells of metabolic organs (Hotamisligil, 2006).

Although these notions are well-established, a comprehensive description of macrophage activation states is not yet available, not to mention the fact that a rational understanding of their functional implications and the underlying mechanisms remain far from being fully characterized. The classical macrophage activation (''polarization'') states M1 and M2 (corresponding to inflammatory macrophages induced by interferon-γ [IFN-γ] and alternatively activated macrophages induced by interleukin-4 [IL-4], respectively) (Gordon and Martinez, 2010) are in fact useful to describe extreme states toward which macrophages can be driven by stimulation (Biswas and Mantovani 2010). However, as it has been recognized for many years, these two states are insufficient to describe the much broader complexity of stimuli and responses that mark the normal life of a macrophage. Therefore, attempts to systematically explore macrophage activation via transcriptomic and systems biology tools are highly valuable and commendable efforts.

In their study, Xue and coworkers investigated the transcriptional changes triggered in human monocyte-derived macrophages by 28 different stimuli (or their combinations), thus generating almost 300 data sets (Xue et al., 2014). One extreme yet informative example of specificity was the identification of a small