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A New Way to Beat Intestinal Pathogens

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Abstract

In the gastrointestinal tract, the tug of war for iron may provide a new way to vaccinate. Recent work shows that immunizing mice with siderophores (small molecules microbes produce to capture iron) foils pathogen colonization and may instead allow a commensal to expand.

Keywords

siderophore; iron; *Salmonella*; vaccine

Microbes and mammals both require the enzyme cofactor iron. During infection, the competition for iron is so intense that microbial iron acquisition systems are major determinants of virulence. One strategy bacteria and fungi use to acquire iron in the host is to secrete small molecules called siderophores which bind Fe^{3+} with extremely high affinity (association constants can exceed 10^{50}) [1]. Microbes then capture the iron–siderophore complex with a specific membrane receptor and release the iron into the cytosol. Siderophore biosynthesis or utilization can be inhibited by small molecules, which have potential for development as antimicrobials [2]. Another iron-based antimicrobial approach exploits siderophores that have been naturally or synthetically conjugated to antibiotics, tricking microbes into taking up the antibiotic along with the siderophore [3]. A third siderophore-based strategy, priming the host to make anti-siderophore antibodies that may prevent microbes from acquiring iron, has recently taken a giant leap forward [4].

While siderophores are not themselves immunogenic, a siderophore conjugated to an immunogenic carrier protein stimulates mice to produce siderophore-specific antibodies within three weeks [5]. This and other observations suggested that vaccinating against siderophores may confer protection against pathogens that require them. Recently a team of microbiologists and chemists took the next major step [4]. Sassone-Corsi *et al.* conjugated the siderophore enterobactin (Ent) to an immunogenic carrier protein. *Salmonella enterica* requires Ent for virulence in mice [6]. The carrier protein, cholera toxin subunit B (CTB) is an adjuvant that promotes mucosal inflammation [7]. The researchers delivered CTB-Ent or

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CTB alone to mouse nasal mucosal tissue twice, two weeks apart (Figure 1). One week later, significant levels of Ent-specific immunoglobulin A (IgA) antibodies appeared in the feces of CTB-Ent but not CTB immunized mice.

Next, the Sassone-Corsi team [4] examined whether vaccination confers resistance to infection with *Salmonella*. Five to eight weeks after immunization, they pre-treated mice orally with the antibiotic streptomycin to increase susceptibility to *Salmonella* [8]. They then challenged orally with 10^9 bacteria [4]. By the fourth day after infection, the CTB-Ent-immunized mice contained 10-fold fewer *Salmonella* in their intestines. Similar results were obtained in a different mouse line, in mice housed under different conditions and in mice intraperitoneally immunized with CTB-Ent. In addition, the researchers observed an encouraging negative correlation between *Salmonella* intestinal load and levels of anti-Ent IgA in individual mice. Altogether, the data suggest that infection could be reduced by optimizing vaccination, and that antibodies to siderophores may prevent *Salmonella* from capturing enough siderophore-bound iron to thrive in the gut.

The researchers also examined whether gut inflammation or the gut microbiota differ in CTB versus CTB-Ent immunized mice. The basis for this inquiry is the survival advantage that *Salmonella* and other facultative anaerobes have in the inflamed gut due to availability of alternative electron acceptors, such as nitrate and tetrathionate [9]. As expected, histopathology and molecular markers revealed no significant inflammation four days after infection or mock-infection. In contrast, infection resulted in inflammation in both CTB and CTB-Ent immunized mice, suggesting *Salmonella* had access to alternative electron acceptors under both conditions. However, *Salmonella* growth in the gut also requires iron captured by siderophores [6], consistent with the failure of CTB-Ent immunized mice to support *Salmonella* colonization. Instead, it appears that in these mice, commensal *Lactobacillus* species expand upon challenge with *Salmonella*. *Lactobacillus* thrive in an inflamed gut for unknown reasons, but do not scavenge enterobactin [10]. These data highlight that in a complex ecosystem, inhibition of one organism, in this case a pathogen, may open up a niche for another organism, in this case, fortunately, a commensal.

In summary, immunization against siderophores can protect the host from a gut pathogen that depends upon siderophores to replicate. This exciting find has broad potential for bacterial and fungal pathogens and merits further study. Key remaining questions are whether IgA antibodies are necessary and sufficient for protection and whether natural transmission of the pathogen is reduced, as anticipated. In addition, it is important to determine whether microbes have the capacity to acquire or evolve resistance to anti-siderophore antibodies. Nevertheless, anti-siderophore vaccines have tremendous potential because they could minimize the spread of siderophore-requiring pathogens in food animals and in people.

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Figure 1. Siderophore Immunization and Challenge Strategy
At zero and two weeks, control CTB or siderophore-conjugated CTB (CTB-Ent, approximately four Ent molecules per CTB) was delivered to mice intra-nasally (100 µg/ mouse) by Sassone-Corsi *et al.* [4]. After three weeks, IgA antibodies specific for siderophores were observed in the feces of CTB-Ent immunized mice, but not in CTB immunized mice. After five to eight weeks, the mice were challenged in a model of *Salmonella* colitis, which requires pre-treatment with the antibiotic streptomycin [8], followed by inoculation with 10⁹ streptomycin-resistant *Salmonella*. Four days later, at the peak of inflammatory response and iron limitation, tissue colonization, body weight, and the gut microbiota were compared.