

# Nutritional immunity with metals

By Dr. Kevin Waldron, Newcastle University, UK

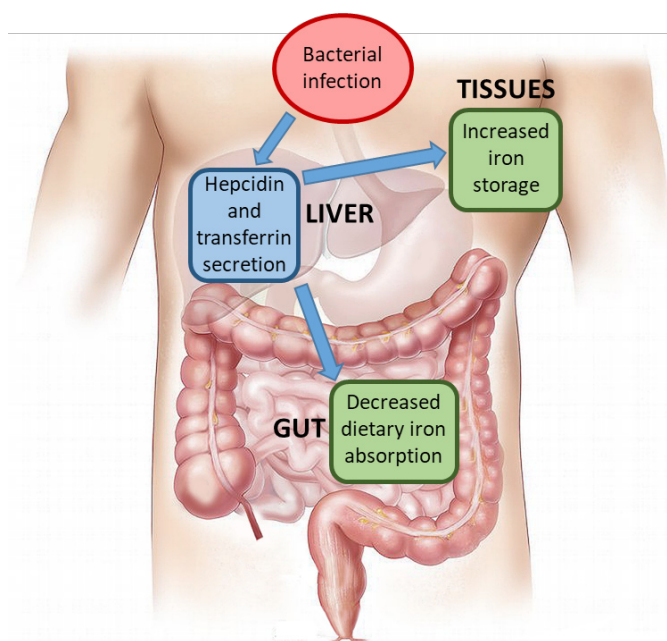
The spread of antibiotic resistance among disease-causing (pathogenic) bacteria is causing increasing consternation among medical and veterinary professionals, researchers, and policymakers worldwide. As a result, there is regulatory pressure to significantly reduce antibiotic usage, both in human medicine and in agriculture, in an effort to control the worrying spread of antibiotic resistance among bacteria. Simultaneously, both academic and industrial researchers are being encouraged to find new, innovative ways to treat future infections. In order to produce such novel antibiotics of the future, much current research is aimed at better understanding how pathogenic bacteria are naturally controlled by the mammalian immune system, in the hope that novel therapeutic strategies can be designed that mimic or exploit these processes.

To successfully establish an infection, once inside the host organism an invading pathogenic microbe must obtain all of its nutritional requirements directly from the host. This creates an opportunity for the immune system to try to limit the pathogen's growth by making it difficult for the microbe to acquire a sufficient supply of essential nutrients. This immune strategy is called 'nutritional immunity'.

An effective use of this strategy by the immune system involves enforcing strict control of the availability of trace minerals during infection. The same metal ions (iron, zinc and manganese, for example) are essential micronutrients for both bacteria and mammals, in which they are exploited as critical cofactors that enable enzymes to catalyse chemical reactions. This creates a competition for these metals at the host-pathogen interface. Their essential nature has led to the evolution of complex metal homeostasis systems in all cells that precisely coordinate metal acquisition, delivery to metal-requiring proteins, and detoxification of excess metal concentrations through storage or export.

## The iron-withholding response

The concept of 'nutritional immunity' is not new. It has been long established that the mammalian immune response to a bacterial infection alters host iron homeostasis in ways that restrict the ability of the invading bacteria to acquire this trace mineral. When an infection is detected by the immune system, a series of signal cascades result in: (i) decreased iron uptake from the intestine into the bloodstream; (ii) increased expression of the iron-transporting protein transferrin in the blood, which coordinates the circulating iron so tightly that most bacteria struggle to compete; (iii) increased storage of iron within host cells to make it more difficult for bacteria in the blood to access this precious resource; and (iv) production of immune proteins that function to interfere with bacterial iron acquisition systems. This global iron response is mediated by the hormone hepcidin, whose synthesis in the liver is influenced by immune signalling (Figure 1).



**Figure 1: The iron-withholding response of the mammalian immune system.**

*Schematic diagram illustrating how, in response to infection and inflammation, bodily iron homeostasis is altered to make this essential trace mineral less bioavailable to the invading microbe. Intestinal iron absorption is decreased, iron storage in tissues is increased, and circulating iron in the blood is protected by induction of the iron-sequestering protein transferrin. These changes are regulated systemically by the hormone, hepcidin.*

## The central role of calprotectin in nutritional immunity

This long-established concept has, however, been greatly extended over the last decade to incorporate immune responses that regulate the availability of other essential metal ions in response to bacterial infection. The central player in this wider metal-depriving response is calprotectin, a protein complex that is produced and secreted in very large quantities, primarily by a specific type of immune cells called neutrophils, once activated through detection of bacterial pathogens. Calprotectin exhibits extremely tight binding affinities for a range of metals, including manganese, zinc and iron, all of which are essential in biology, and there is accumulating evidence that calprotectin can influence the competition for each of these metal ions at the host-pathogen interface.

The role of calprotectin in nutritional immunity was first discovered through study of its role in skin and soft tissue infections caused by the Gram positive bacterium, *Staphylococcus aureus*. This organism has gained infamy through the spread of strains that

are resistant to the antibiotic methicillin, termed MRSA (methicillin resistant *Staphylococcus aureus*). The calprotectin protein was detected to accumulate at the site of *S. aureus* infection, termed an abscess, in infected mice. The purified protein was shown to be an effective inhibitor of the growth of *S. aureus* in the test tube, and the mechanism of this inhibition was demonstrated to be through the tight coordination of manganese ions by the calprotectin complex. This in turn leads to manganese starvation of *S. aureus* cells when they are in the presence of calprotectin, whether in laboratory experiments or inside abscesses within the infected host. It was subsequently shown that manganese starvation resulted in a reduced ability of *S. aureus* to supply an essential enzyme, called superoxide dismutase, with its essential manganese cofactor. This enzyme is known to be crucial in defending the bacterium against a key weapon utilised by the mammalian immune system to kill invading bacteria.

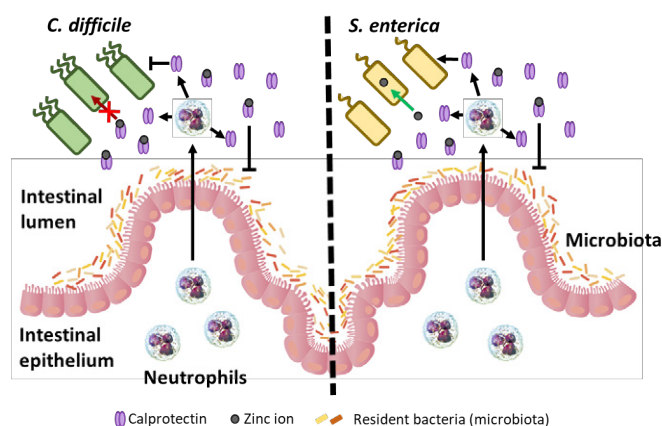
### Nutritional immunity in the intestine

It is important to note that the calprotectin is known to play a number of important roles in the body, some of which are unrelated to its function in control of bacterial pathogens. This makes it an unreliable biomarker of inflammation. Nonetheless, calprotectin is also an important effector in the immune response to other pathogens, including those that cause infection through the colonisation of the mammalian gut. Cells of the intestinal epithelium also produce calprotectin, although notably the primary source of calprotectin in the intestine remains the neutrophil cells of the immune system, which infiltrate the gut lumen during the inflammatory response caused by the detection of pathogenic bacteria within the intestine. However, it's notable that studies of the influence of calprotectin on bacterial infections within the mammalian intestine have found that zinc, rather than manganese, is the key metal whose availability is affected by the presence of calprotectin in this niche. Interestingly, this sequestration of intestinal zinc by secreted calprotectin can have dramatically different effects on the outcome of bacterial infection of the gut, depending on the bacterial pathogen that is encountered (Figure 2).

Intestinal infection by the human pathogen *Clostridium difficile* exhibited a classical nutritional immunity response to the presence of calprotectin. It was shown that the growth of *C. difficile* is strongly inhibited by calprotectin in laboratory experiments. This inhibition is caused by zinc starvation of the bacterium through tight coordination of the available zinc by the calprotectin complex. An analogous effect was observed during infection of mice with *C. difficile*; calprotectin secreted into the gut lumen inhibited bacterial growth and proliferation, whereas mice that were genetically modified to prevent them from being able to synthesise calprotectin were found to be especially vulnerable to succumbing to intestinal infection by *C. difficile* relative to wild type mice.

Like *C. difficile*, growth of the Gram negative pathogenic bacterium *Salmonella enterica* serovar Typhimurium was also found to be inhibited by the presence of calprotectin in the test tube, but the degree of growth inhibition was observed to be significantly less than

the inhibition of *C. difficile*. Crucially, however, the effect of calprotectin on *Salmonella* infections in mice were the opposite of that observed in the experiments with *Clostridium* infections; the presence of calprotectin appeared to increase the ability of *Salmonella* to establish infection in wild type mice. It was established that this difference was caused by the presence of a high affinity zinc acquisition system in *Salmonella*, which is able to compete with calprotectin for the available zinc. This zinc uptake system is absent from the genome of *C. difficile*. Mutant strains of *Salmonella* in which this zinc uptake system was abolished showed significantly decreased ability to colonise the mouse gut, and were hugely disfavoured relative to wild type *Salmonella* during competitive infection of wild type mice, but not in strains of mice that were unable to produce calprotectin. This indicates direct competition between the *Salmonella* zinc acquisition system and host calprotectin for zinc ions within the gut. Therefore, this high affinity zinc acquisition system enable *Salmonella* to compete against calprotectin, and thereby maintain its zinc supply to essential zinc-requiring enzymes under the calprotectin-mediated zinc limiting conditions in the lumen of the inflamed intestine. Furthermore,



**Figure 2: Nutritional immunity has different effects on different pathogens inside the intestine.**

Schematic diagram illustrating how the nutritional immunity effector, calprotectin, affects the ability of the pathogenic bacteria (left) *Clostridium difficile* and (right) *Salmonella enterica* serovar Typhimurium to establish infection of the mammalian intestine. During inflammation, neutrophils invade the gut lumen and release calprotectin, which tightly coordinates metal ions in the lumen, most notably zinc. The very tight binding of zinc to calprotectin reduces the bioavailability of zinc, both to the resident bacteria of the microbiota and also to the pathogen *C. difficile*, which is unable to effectively compete against calprotectin. This results in *C. difficile* zinc starvation, making calprotectin an effective inhibitor of *C. difficile* infections. Conversely, *Salmonella* cells possess a high affinity zinc acquisition system that can more effectively compete against calprotectin for zinc, ensuring its continued access to this essential micronutrient. Calprotectin does, however, inhibit the ability of the microbiota to acquire zinc, thus calprotectin acts to inhibit their ability to protect the gut from colonisation by the pathogen.

*Salmonella* also exploits the fact that calprotectin depletes zinc availability to other bacteria of the natural gut microbiota (the natural flora of diverse bacteria that are present inside the gut that provide protective and metabolic functions beneficial for the host organism) that are present in the intestinal lumen; *Salmonella* thrives while its competition struggles to overcome the zinc starved conditions.

### Perspectives

As a relatively new research field, it is notable that most primary studies in this area have been performed on pathogenic bacteria of human medical significance and have utilised mice as the experimental host. Nonetheless, these studies have established the importance of nutritional immunity in fighting pathogenic bacteria, identified key host proteins that are involved in the process, and also determined some of the mechanisms by which certain pathogens are able to circumvent nutritional immunity. These studies have also demonstrated that proteins such as calprotectin can have different effects on the homeostasis of different metals within different bodily niches that are of relevance to different pathogenic bacteria. However, few attempts have been made to extend this concept to other animal

systems. Notably, farm animals are routinely supplied with excess quantities of trace minerals in their diet, which have been established to provide beneficial effects on animal physiology, although the mechanisms of these protective benefits remain unclear. This dietary metal excess is in stark contrast with the situation in humans, whose diets are routinely metal-deficient, especially in the developing world. Further research is urgently needed to determine how these nutritional immunity mechanisms operate in agriculturally important animals, and how their influence on gut health is affected by this supplementation of animal feed with excess metals such as zinc. The examples illustrated above, however, are likely to be indicative; the zinc transport systems are highly conserved between *Salmonella* and *Escherichia coli*, one of the primary Gram negative pathogens that cause significant damage to animal health and to the meat production industry, as are the zinc transporters of the human pathogen *C. difficile* and *Clostridium perfringens*, another significant pathogen of animals. Future studies should aim to address this knowledge gap.

*This article is linked to the presentation made by Kevin WALDRON at the 3rd Animine Academy - September 2019*