# Anything You Can Do, I Can Do Better

For the most part, the commensal bacteria, and the humans on which they live, live in a balanced symbiotic relationship. However, the bacteria may, if the circumstances are right, take the form of opportunistic pathogens e.g. a minor number of *Escherichia coli* strains are the common causes of urinary tract infections, which can develop further to septicaemia if not treated adequately (Ron 2010). Another opportunistic pathogenic commensal is *Staphylococcus aureus*, which may also cause a systemic infection if it breaches the normal colonisation sites (Stauff, Bagaley et al. 2008). In order for such opportunists to be successful in causing systemic infection, they must fulfil 2 requirements: 1) they must be able to survive in the serum, which is full of bactericidals such as immune cells and the complement proteins, and 2) they must be effective at gaining nutrients, particularly iron, from the host (Ron 2010, Stauff, Bagaley et al. 2008, Anzaldi, Skaar 2010). Failure to perform these 2 tasks almost invariably denotes a failure in the bacterium's ability to systemically infect and survive.

Iron is essential for literally all organisms, for metalloenzyme use, and Fe-S clusters in redox and metabolism, in protection against oxidative stress, and also in immunity by promoting T cell clonal proliferation, and ROS production in phagocytes for use in phagosomes (Ron 2010, Anzaldi, Skaar 2010, Jones, Niederweis 2011, Nairz, Schroll et al. 2010). Iron is not readily available in the human body for invading pathogens to use, with 70% of the total iron being contained in haem groups in haemoglobin (Hb). Most of the rest is either stored intracellularly in ferritin, or is bound to transferrin in the serum or is 'free' (Anzaldi, Skaar 2010, Nairz, Schroll et al.

2010). During infection, the immune system releases a range of proteins, mainly transferrin and lactoferrin, from neutrophils and tissues such as the kidney, which bind to any 'free iron,' and are then internalised by $M_2$ macrophages, sequestering iron (Abrink, Larsson et al. 2000), and driving the serum iron concentration to below $10^{-24}$M, far too low for bacteria to survive (Nairz, Schroll et al. 2010, Beasley, Heinrichs 2010). This process also occurs following intravascular haemolysis, which can be induced by pathogens (Anzaldi, Skaar 2010, Nairz, Schroll et al. 2010). In addition to this, iron can be pumped out of infected cells, e.g. macrophages in *Mycobacterium tuberculosis* infection, by upregulating Fpn1 expression, an efflux pump, to reduce iron availability to the pathogen (Nairz, Schroll et al. 2010).

Bacteria unable to obtain iron from the host are typically non-pathogenic, and as such many virulent strains have evolved various methods to acquire this required resource (Weinberg 1974). One of the most common ways is by siderophore production, such as aerobactin from *E. coli* on the ColV plasmid, staphyloferrin-A and staphyloferrin-B from *S. aureus*, and *M. tuberculosis* carboxymycobactins (Ron 2010, Nairz, Schroll et al. 2010, Beasley, Heinrichs 2010). These are small electronegative, high affinity ferric iron scavengers which are internalised via ATP-binding cassette (ABC) transporters by the bacteria (Ron 2010, Beasley, Heinrichs 2010). However, thinking about it logically, it is a rather useless method of iron acquisition, as most of the iron in the body is bound or stored away. To circumvent this, some bacteria have modified their siderophore methods. For example, staphyloferrin-B, from *S. aureus*, actually leeches iron from transferrin itself, and the bacterium also has a wide variety of siderophore receptors, including for exogenous siderophore such as Fhu-D1-D2 for hydroxamate siderophores, utilising all the iron it

can (Beasley, Heinrichs 2010). Lipocalin-2 is another secreted protein in response to infection, which binds to siderophores, preventing their action, but *M. tuberculosis* post-translationally modifies its caroxymycobactins, making them unrecognisable to the protein. Another immune response to *M. tuberculosis* infection is expression of Slc11a1, a transmembrane protein, which reduces available iron in the phagosome, but expression of this modulator causes the bacterium to upregulate its caroxymycobactins, acquiring what iron is available, and also halting the maturation of the phagosome (Nairz, Schroll et al. 2010).

Despite these evolutionary steps forward, the bacterial use of siderophores is not the most effective way of obtaining iron, as 70%/2.5mg of iron is stored in Hb. The next development in bacterial virulence, in the context of iron nutrition, is shifting from bacterial acquisition of free iron, to haem iron. There are 3 main systems that bacteria use to do this, which are all present in GRAM negative bacteria. The first is a direct haem uptake, used in *Pseudomonas aeruginosa*, which involves a TonB-like complex (PhuR) utilising a proton motive force to transport haem to the periplasm, where it is bound by haem transport proteins (PjuT), and is then internalised via ABC transporters (PhuUVW). *Neisseria* spp. are the only known users of bipartite intake, which involves a dimeric complex outer membrane receptor, HpuAB, with HpuA resembling the TonB complex in direct uptake. How the haem group is transported across the inner membrane in bipartite uptake systems is unknown. The final method is similar to the siderophores, but instead haemophores are secreted, such as HasA in *P. Aeruginosa*, which bind haem, and are internalised via TonB-like complex receptors (Tong, Guo 2009).

GRAM positive bacteria are much less diverse than the GRAM negative bacteria in terms of the methods they use to obtain haem. Most of them use a direct haem uptake system, similar to the GRAM negative pathogens, with surface exposed haem receptors, cell wall transporting proteins and a memb rane ABC transporter. A good example is *S. aureus* which uses the *i*ron-regulated *s*urface *d*eterminant (*isd)* operon, consisting of 10 genes. IsdB binds to Hb, which then transfers Hb to IsdA (aided by IsdH), and then to IsdC (Tong, Guo 2009). IsdC uses HtsABC, an ABC transporter, and haem oxygenases, IsdI and IsdG, to internalise the haem and release the iron within, following haemolysin -mediated haemolysis (Stauff, Bagaley et al. 2008). *Bacillis anthracis* is the only GRAM positive bacterium known to use a haemophore system, in which the haemophores are IsdX1 and IsdX2, and the haem is internalised following binding via the direct system just described (Tong, Guo 2009). *M. tuberculosis* also has been shown to be able to utilise haem by haem degrading enzymes, although its ability to halt phagosome maturation, and thus its intracellular proliferative potential, is dependent on siderophores. How it internalises haem is unknown, but due to the size of haem -conjugated complexes, it must use machinery, as diffusion would be too slow (Jones, Niederweis 2011). Evolution of strains, enabling the bacteria to utilise haem provides a much larger source of iron for the invading pathogen, enhancing its virulence, but it is also more advantageous for some bacteria, such the *Streptococci* and other lactic acid bacteria , which incorporate the haem into their partially complete respiratory chains, enabling them to tolerate ROS and use $O_2$. This results in increased biomass and long term stability (Pessione, Lamberti et al. 2010, Lechardeur, Cesselin et al. 2011) . Some bacteria, such as the alphaproteobacteria, are capable of producing their own haem,

removing dependency on the host, which is an optimum position to be in, for highly virulent pathogens (Tong, Guo 2009).

This virulent advance to haem acquisition brought with it a hazard for the bacteria though. Haem in great enough concentrations is very toxic (Lechardeur, Cesselin et al. 2011), causing a hindrance of growth and potentially cell death, most likely via ROS induced DNA damage (Stauff, Bagaley et al. 2008, Everse, Hsia 1997). Thus, these virulent pathogens must control internal haem as well as be able to acquire it. There are 2 main methods used to do this. The first is sequestion of haem by cytoplasmic proteins, such as ChiS in *E. coli O157:H7*, the presence of which is a strong indicator of virulence (Tong, Guo 2009). The other method is the regulation of contained haem, either by increased export of haem or metabolites, or by degradation of toxins. Haem oxygenases are well used in degradation producing the non-toxic biliverdin from excess haem. However, the most common method appears to be the use of efflux pumps. In fact, *Streptococcus agalactiae* uses 2 efflux systems. These are pefAB and pefCD from the *pef* operon, which expel haem and protoporhyrin IX respectively, constitutively, and also the HrtAB pump, which is used only in high haem concentrations, probably a development for self-preservation and protection (Fernandez, Lechardeur et al. 2010). HrtAB was discovered in *S. aureus* primarily, which, along with *B. anthacis*, also uses an intricate 2 component haem sensing cascade, HssRS. In the presence of haem, this induces HrtAB expression and ABC efflux of toxic haem occurs (Stauff, Bagaley et al. 2008, Tong, Guo 2009). Mutations in any of these pumps, enzymes, sensors, receptors result in a loss of ability to tolerate haem, a loss ability of survive in the haem-filled blood, and a loss of virulence (Tong, Guo 2009).

It can be seen why iron is so important for growth generally, and especially for virulent, systemic pathogens. Its transitional capabilities make it a suitable metal for catalytic or co-enzymatic use, and thus it is not surprising that the human immune system, the iron storage system design itself, has developed to hide all the iron that it can away from any invaders. Yet, considering its importance to the bacteria as well, the clear progression from obtaining free iron to obtaining protein-conjugated iron e.g. transferrin, to accessing the largest iron store of all, haem groups, is also not surprising. The virulent bacteria which cause systemic infections have adapted very well to the environment that the human body provides, in order to access the body's iron, a key nutrient vital to their survival.

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