Iron and infection: better understanding at the molecular level but little progress on the clinical front

The topic of iron and infection originates in the mid-1940s with the work of Schade and Caroline on the bacteriostatic properties of egg white and plasma. Although largely ignored for over 20 years, their observations were responsible indirectly for renewed interest in the late 1960s when animals given injections of iron in various forms were found to be more susceptible to bacterial infection than untreated controls. However, the interpretation of results from in-vivo experiments is often difficult and the relevance of these experiments to human clinical medicine has been vigorously debated. Following intensive research, especially at the molecular level, the subject has at last become scientifically respectable and iron is now recognised as playing an important role in infection. Its importance lies in the strictly limited availability of the metal in living tissue. Normal body fluids contain high-affinity iron-binding glycoproteins, transferrin or lactoferrin, both of which bind iron tightly and ensure that no free iron is available to invading bacteria.

Progress in understanding the strategies used by pathogens for acquiring iron in vivo, and their responses to iron restriction, has provided fresh insight into microbial pathogenicity. As is now well appreciated, many bacteria respond to iron restriction by producing siderophores together with their membrane receptors; these systems are responsible for acquiring iron from iron-binding proteins. In some cases the role of siderophore-mediated iron-uptake systems in virulence is clear. For example, highly virulent strains of *Vibrio anguillarum*, which are responsible for a devastating septicaemic disease of fish, carry a plasmid encoding a siderophore-dependent iron-transport system that allows the organism to grow under iron-restricted conditions. On losing the plasmid, *V. anguillarum* loses both its ability to grow under such conditions and its virulence. In other cases, the contribution of siderophores to virulence is less obvious. The production of the *V. cholerae* siderophore, vibriobactin, seems not to be essential for infection of intestinal mucosae, although there is evidence to suggest that at least some of the iron-regulated membrane proteins are switched on in vivo. Part of the difficulty in assessing the role of siderophores in natural infection is often related to the type of animal model used. Furthermore, other iron-uptake systems may operate in vivo, and it has been suggested that traces of haem or haemoglobin could serve as a source of iron for *V. cholerae*. Some pathogens move to intracellular locations where iron seems to be more available. An increasingly reported phenomenon is the ability of pathogens to use siderophores produced by other micro-organisms but which they themselves are unable to synthesise. Ferrioxamine, for example, is produced by *Streptomyces* spp. but is able to supply iron to *Klebsiella* and *Salmonella* spp. and *Yersinia enterocolitica*. Since the methane sulphonate salt of desferrioxamine B (Desferal, Ciba-Geigy) is widely used clinically in deferration therapy, it might be expected to increase the susceptibility of treated individuals to certain bacterial infections by making iron more readily available, and indeed cases of septicaemia due to *Y. enterocolitica* in individuals treated with Desferal have been reported. Whether the ability to use exogenous siderophores plays any part in natural infections, remains unproven. Recently, interest has arisen in the additional contribution siderophores might make to the pathogenesis of infection by suppressing host immune responses and promoting inflammation and tissue damage at sites of infection through siderophore-induced free radical formation. Depending upon their biochemical properties, siderophores behave differently in such systems, and this may lead to different clinical consequences.

Not all pathogens produce siderophores. Some, notably *Neisseria* spp. and *Haemophilus influenzae*, express membrane proteins under conditions of iron restriction which act as receptors for transferrin or lactoferrin, the iron being acquired by direct interaction between the iron-binding protein and the bacterial cell surface. These mechanisms are distinguished from siderophore-mediated iron uptake mechanisms by their high specificity. For example, the iron uptake system of *N. meningitidis* is highly specific for human transferrin or human lactoferrin. This may help explain host specificity and has obvious implications for the development of animal models. Although details of the molecular mechanisms of iron-uptake remain unclear, progress is being made in identifying and characterising lactoferrin- and transferrin-binding proteins. The amino-acid sequences of some transferrin-binding proteins, together with preliminary data on the organisation of the encoding genes, have recently been reported (and at the 8th International Pathogenic Neisseria Conference, Mexico, October 1992) and should allow rapid progress towards identification of the transferrin-binding
site. Conserved antigenic regions have also been found amongst some of these proteins from different pathogens and there is considerable interest in their vaccine potential, especially as components of vaccines against serogroup B meningococci for which no entirely effective human vaccine has yet been developed. Over the past few years it has become evident that the restricted availability of iron in vivo not only presents microbial pathogens with the problem of acquiring sufficient for multiplication, but also constitutes a major environmental signal which regulates the expression of a number of virulence genes unrelated to iron metabolism, such as those encoding toxins. In Escherichia coli, the expression of many iron-controlled systems is negatively regulated via a global repressor protein Fur, which uses Fe⁺⁺ as a corepressor. Binding sites for Fur, at a sequence called the “iron-box”, have been identified in several iron-regulated promoters and Fur-like regulatory systems have been found in several pathogens, including Corynebacterium diphtheriae where iron regulates the synthesis of diphtheria toxin.

Whilst considerable advances are being made in understanding the molecular basis of iron-related virulence processes, less progress has been made in understanding the clinical consequences of increased iron-availability and this continues to be a controversial topic. The abnormal presence of freely available iron in vivo would be expected to increase the rate of bacterial multiplication and to tip the balance in favour of the invading pathogen. There is also evidence that iron interferes with the activities of some antibacterial systems. Some authors cite clinical situations to support the importance of iron availability in determining infection whereas others quote alternative, sometimes rather ill-defined, explanations of the same data, and remain sceptical of the importance of iron in host resistance. In reality, the factors that decide the outcome of infections are numerous and complex, and it is not surprising that it is often difficult to establish the relative contribution of each.

There is no doubt that the availability of iron can, in some circumstances, have an important influence on the clinical outcome of infections. For example, freely available haem or haemoglobin clearly promotes infection and proposals to use cross-linked haemoglobin as red-cell substitutes in transfusion medicine should be very carefully evaluated; any advantages these products might have over whole blood could be outweighed by the promotion of bacterial proliferation in vivo, and an increase in the susceptibility of recipients to infection. However, further research is required into the effect of changes in host iron metabolism and transferrin saturation levels on the susceptibility of compromised individuals to infection.

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References