Host range and tissue colonization of pathogens: role of iron acquisition

Weinberg, E.D.

Department of Biology, Indiana University, Bloomington, IN 47405

ABSTRACT

In order to replicate, invading pathogens must gain access to host iron. Vertebrate hosts have developed an extensive iron withholding defense system that, while providing essential iron to their own cells, attempts to deprive invaders of the metal. To obtain host iron, successful pathogens employ one or more of four strategies: (i) erythrocyte lysis, hemoglobin digestion, and heme assimilation; (ii) binding of ferrated siderophilins with extraction of iron at the cell surface; (iii) production of siderophores that withdraw iron from transferrin; and (iv) procurement of host intracellular iron. Each strategy contains much variation. Individualized strategies are important determinants of host range of the pathogens as well as of their localization and growth in specific tissues and cells.

During the past third of a century, several thousand published reports of clinical observations and laboratory investigations have described the crucial role of iron acquisition in the establishment of infection and neoplasia in animals and humans [12,13]. Only the most virulent of microbial and neoplastic strains are capable of acquiring growth-essential iron from healthy hosts; the latter possess an elaborate iron withholding defense system (Table 1). Disease due to highly virulent strains is exacerbated in potential hosts whose iron withholding defense is compromised (Table 2). Such hosts may also suffer serious illness as a result of invasion by less virulent strains that have lesser ability to capture iron.

An illustrative example of the critical importance of iron in determining the outcome of a microbial invasion in humans is that of Legionnaire's pneumonia. The pathogen is a gram negative bacterium termed *Legionella pneumophila*. In its natural aquatic habitat, the bacterium survives by growing within a variety of saprophytic protozoa. It enters humans upon inhalation of contaminated water droplets. *Legionella pneumophila* can successfully evade human host defense only by parasitizing and multiplying within alveolar macrophages. Seven lines of evidence summarized below indicate that the ability of *L. pneumophila* to multiply in the patient is dependent upon macrophages that are iron-loaded.

First, *Legionella pneumophila* cannot obtain iron from natural extracellular environments. For growth in pure (artificial) culture, the bacterium requires more than twenty-fold the amount of iron that is needed by cells of other microbial species, plants, or animals [9]. Additionally, a reducing agent such as L-cysteine must be included in the culture medium.

Second, human monocytes treated with such iron chelators as deferoxamine (DFO), apotransferrin, or apolactoferrin do not support multiplication of *L. pneumophila* unless

Microbial Biosystems: New Frontiers
Proceedings of the 8th International Symposium on Microbial Ecology
Bell CR, Brylinsky M, Johnson-Green P (ed)
Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

Table 1. Selected aspects of the iron withholding defense system.[a]

Constitutive components:

1. Siderophilins
   Transferrin in plasma; lymphatic and cerebrospinal fluid
   Lactoferrin in tears, milk, and secretions of the respiratory, gastrointestinal, and genital tracts
2. Ferritin within host cells

Processes induced at time of microbial or neoplastic cell invasion:

- Suppression of assimilation of dietary iron[b]
- Suppression of iron efflux from macrophages that have ingested hemoglobin of effete erythrocytes[b]
- Synthesis of additional ferritin to sequester retained iron[b]
- Suppression of expression of transferrin receptors of invaded cells[c]
- Synthesis of nitric oxide (from L-arginine) to impair iron metabolism of
- intracellular pathogens, helminth pathogens, and cancer cells[c]
- Synthesis of immunoglobins to microbial cell surface iron acquisition proteins

[a]modified with permission from Table 1 of [12]

[b]activated by interleukin-1 or –6, or by tumor necrosis factor alpha

[c]activated by interferon gamma

Table 2. Factors that can impair the iron withholding defense system and increase the risk of infection and neoplasia.

Excessive assimilation of ingested iron	
	African siderosis, alcoholism, asplenia, folic acid deficiency, hemochromatosis, porphyria cutanea tarda, thalassemia
Ingestion of excessive amounts of iron	
	Iron-adulterated processed foods
	Milk formula loaded with high iron
	Red meats
	Supplements laced with iron
Injection of excessive amounts of iron	
	Iron saccharates*
	Packed erythrocytes*
	Whole blood*
Inhalation of iron	
	Asbestos (amosite, crocidolite, tremolite)
	Ferriferous minerals
	Tobacco smoke
	Urban air particulates
Decompartmentalization of iron	
	Hemolytic conditions
	Hepatitis

*in absence of unequivocal medical justification

excess iron is provided [6]. Third, interferon gamma suppresses growth of *Legionella pneumophila* in human monocytes by reducing host cell expression of Fe-transferrin receptors [6].

Fourth, chloroquine suppresses *Legionella pneumophila* growth by raising the acidic pH value of the host cell endosome, thereby preventing Fe-transferrin from releasing the metal to the bacterium [3]. Fifth, murine peritoneal macrophages support growth of *Legionella pneumophila* if loaded with extra iron [5]. Sixth, iron-defective mutant strains of *Legionella pneumophila* have lost pathogenicity as well as ability to grow in saprophytic protozoa [8].

Seventh, humans who are most susceptible to Legionnaire's pneumonia are smokers. Tobacco contains 440-1150 ug iron/g and cigarette paper contains 420 ug iron/g. Approximately 0.1% of cigarette iron is contained in the mainstream smoke. A one pack per day smoker thus can inhale several million picograms of iron per day. The iron burden of alveolar macrophages was found to have increased threefold in persons who have smoked 50 pack-years, and 5.4-fold in those who smoked 100 pack-years, relative to non-smokers [11].

Other obligate intracellular pathogens likewise have been observed to require iron for replication. In each case, host cell defense involves iron depletion. For example, *Chlamydia trachomatis* growth in human epithelial cells was suppressed by interferon gamma or by deferoxamine [10], and enhanced by both Fe-transferrin [10] and analogs of L-arginine [7]. Macrophages infected with *Ehrlichia chaffeensis* likewise were protected by interferon gamma or DFO; the protective action of the cytokine was reversed by Fe-transferrin [1]. Moreover, *E. chaffeensis* has been observed to upregulate host cell expression of transferrin receptor mRNA [2]. Prevention of nitric oxide synthesis by administration of monoclonal antibodies to interferon gamma or by administration of L-arginine analogs allowed sub-lethal strains of *Rickettsia conori* to kill 100% of test animals [4].

Examples of determination of tissue sites of infection and of host range as functions of iron acquisition capability are presented in Table 3.

Table 3. Association of microbial iron acquisition mechanisms with specific host susceptibilities and with tissue and cell locations [13].

Microbial employment of siderophilin receptors

Features:

Very narrow host range; pathogens can cause disease only in hosts that have corresponding siderophilins; antibodies to microbial receptors protect hosts from reinfection

Examples:

Neisseria meningitidis and *Helicobactor pylori* in humans;
Hemophilus somnus and *Pasteurella hemolytica* in cattle;
Actinobacillus pleuropneumoniae in swine

Microbial employment of siderophores to extract iron from siderophilins

Features:

Can often be pathogenic in more than one host species

Example:

Listeria monocytogenes. Grows in mammals, birds, fish, and crustacea. Forms no siderophores itself, but employs molecules found in hosts such as human catecholamines. The invader has a cell surface ferric reductase that apparently recognizes chelated iron sites on xenosiderophores; the metal is then reduced and assimilated.

Host sites of infection as a function of iron acquisition capability

Helicobacter pylori:
Binds human Fe-lactoferrin in gastric mucosa and binds heme in intercellular junctions of epithelial cells.

Salmonella typhimurium:
Forms siderophores for extracellular growth in gut; none needed for intracellular growth; siderophore-minus strains are pathogenic if injected but not if fed orally

Shigella flexneri:
Forms siderophores for growth in gut lumen; none needed for growth within epithelial cells

Yersinia pestis:
In normal hosts, hemin-minus strains are pathogenic if injected, but not if fed orally. In iron-loaded hosts, hemin-minus strains are pathogenic either if injected or if fed orally.

References

1. Barnewall RE, Rikihisa Y (1994) Abrogation of gamma interferon-induced inhibition of *Ehrlichia chaffeensis* infection in human monocytes with iron transferrin. Infec Immun 62:4804-4810
2. Barnewall RE, Rikihisa Y (1997) *Ehrlichia chaffeensis* inclusions are early endosomes which selectively accumulate transferrin receptor. Infec Immun 65:1455-1461
3. Byrd TF, Horwitz MA (1991) Chloroquine inhibits the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron. J Clin Invest 88:351-357
4. Feng H-M, Popov VL, Walker DH (1994) Depletion of gamma interferon and tumor necrosis factor alpha in mice with *Rickettsia conori*-infected endothelium: impairment of rickettsial nitric oxide production resulting in fatal, overwhelming rickettsial disease. Infec Immun 62:1952-1960
5. Gebran SJ, Newton C, Yamamoto Y, Widen R, Klein TW, Friedman H (1994) Macrophage permissiveness for *Legionella pneumophila* growth modulated by iron. Infec Immun 62:564-568
6. Horwitz MA (1992) Interactions between macrophages and *Legionella pneumophila*. Curr Top Microbiol Immunol 181:265-282
7. Mayer J, Woods ML, Vavrin Z, Hibbs JB, Jr (1993) Gamma interferon-induced nitric oxide production reduces Chlamydia infectivity in McCoy cells. Infec Immun 61:491-497
8. Pope CD, O'Connell WA, Cianciotto NP (1996) *Legionella pneumophila* mutants that are defective for iron acquisition and assimilation and intracellular infection. Infec Immun 64:629-636
9. Quinn FD, Weinberg ED (1988) Killing of *Legionella pneumophila* by human serum and iron-binding agents. Curr Microbiol 17:111-116
10. Raulston JE (1997) Response of *Chlamydia trachomatis* serovar E to iron restriction in vitro and evidence for iron-regulated chlamydial proteins. Infec Immun 65:4539-4547
11. Weinberg ED (1993) Association of iron with respiratory tract neoplasia. J Trace Elem Exper Med 6:117-123
12. Weinberg ED (1995) Acquisition of iron and other nutrients in vivo. In: Roth JA, Bolin CA, Brogdon KA, Wannemuehler MJ (eds) Virulence mechanisms of bacterial pathogens. Amer Soc Microbiol, Washington, DC, pp. 79-94
13. Weinberg ED (1998) Patho-ecologic implications of microbial acquisition of host iron. Rev Med Microbiol 9:171-178.