HHS Public Access

Author manuscript

Curr Opin Microbiol. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Curr Opin Microbiol. 2015 October ; 27: 57–61. doi:10.1016/j.mib.2015.07.005.

Transferrin-Mediated Iron Sequestration As a Novel Therapy for Bacterial and Fungal Infections

Kevin W. Bruhn[1] and **Brad Spellberg**[2]

[1]Department of Microbiology & Immunology, Keck School of Medicine at the University of Southern California (USC), Los Angeles, CA

[2]Department of Medicine, Keck School of Medicine at USC, Los Angeles, CA

Abstract

Pathogenic microbes must acquire essential nutrients, including iron, from the host in order to proliferate and cause infections. Iron sequestration is an ancient host antimicrobial strategy. Thus, enhancing iron sequestration is a promising, novel anti-infective strategy. Unfortunately, small molecule iron chelators have proven difficult to develop as anti-infective treatments, in part due to unacceptable toxicities. Iron sequestration in mammals is predominantly mediated by the transferrin family of iron-binding proteins. In this review, we explore the possibility of administering supraphysiological levels of exogenous transferrin as an iron sequestering therapy for infections, which could overcome some of the problems associated with small molecule chelation. Recent studies suggest that transferrin delivery may represent a promising approach to augment both natural resistance and traditional antibiotic therapy.

Keywords

transferrin; antibiotic resistance; bacteria; fungi; combination therapy

Introduction

In recent years the critical role of iron in microbial growth and pathogenesis has garnered increasing attention. Virtually all microbes must obtain iron in order to survive and propagate [1–5], including disease-causing pathogens that establish infections in mammalian hosts. Many microbes have thus evolved specialized mechanisms to acquire this limited resource. Conversely, an evolutionarily conserved, common host strategy to control microbial growth is to strictly regulate levels of available free iron. The microbial requirement for iron suggests that strategies aimed at blocking iron acquisition by microbes

Corresponding author: Kevin W. Bruhn, PhD, 1441 Eastlake Avenue, NTT 6427, Los Angeles, CA 90033, (323) 865-3737; kbruhn@usc.edu.

Disclosures

The authors have no conflicts of interest.

might form the basis for promising, novel anti-infective therapies. Unfortunately, previous approaches using small molecule iron chelators have not proven safe and effective for treating clinical infections. In this review, we examine the potential of transferrin, a mammalian iron-binding protein, to be developed as a novel therapeutic for bacterial and fungal disease.

Limitations of small molecule iron chelators as therapeutics

The simplest approach to blocking iron acquisition by pathogens is the use of small molecule chelating agents that sequester iron and prevent microbial uptake. Numerous investigators over several decades have characterized a variety of iron sequestering agents that inhibit the growth of microbes *in vitro* [6–10]. However, several critical barriers have limited development of small molecule chelators as therapies for infection. First is the production by bacteria of siderophores (e.g., *S. aureus* staphyloferrin A and B, and *A. baumannii* acinetobactin) that are secreted by the microbe to scavenge and acquire iron in environments where bioavailability is low [11,12]. Siderophores are extremely strong binders to ferric (Fe^{3+}) iron, and often possess iron affinities that are 10^{10}- to 10^{20}-fold higher than small molecule or biological iron chelators [13,14]. Similarly, fungal species such as *Candida* also produce high affinity iron siderophores, and both fungi and bacteria (e.g., *Acinetobacter*) can uptake high affinity xeno-siderophores that are produced by other bacteria (e.g., desferoxamine) [15–21]. The extremely high iron affinities of microbial-derived siderophores, which are far higher than affinities for small molecule chelators, has led to the perception that iron acquisition by high affinity siderophores cannot be overcome *in vivo* by chelation-based therapy.

An additional problem is that small molecule chelators alter metabolic disposition of iron in ways that may be injurious to the host. For example, chelators reduce iron availability to myeloid cells, which are normally the predominant recyclers of iron in the host, and increase its excretion into renal tubules where iron is not normally found. Thus, serious toxicity to bone marrow, kidneys, and other organ systems can occur during small molecule iron chelator therapy [22,23].

As a result of these factors, *in vivo* testing of iron chelation strategies has focused on eukaryotic pathogens (e.g., malaria and molds) [24,25]. Unfortunately, the most advanced effort to develop a small molecule chelator into clinical trials for infection failed, as a recent randomized, controlled trial of patients with mucormycosis found that small molecule iron chelation was not safe or effective [26]. Nevertheless, the profound requirement for iron acquisition of microbes continues to spur translational efforts to develop novel therapies.

Transferrin as an innate immune mediator

Given how fundamental iron acquisition is to microbial survival, it is not surprising that in mammals, the concentration of free iron in tissue fluids is less than 10^{-24} M. This exceedingly low concentration is maintained predominantly by the iron-binding protein transferrin [27]. Transferrin is an abundant serum glycoprotein that mediates transport and homeostasis of iron levels in the plasma and extracellular tissue fluid. The protein contains two homologous lobes, each with a single high-affinity iron-binding site. Average

transferrin levels in the serum are between 1 and 4 mg/mL, and under normal physiological conditions, the protein remains approximately 30% iron-saturated [28]. Normal levels of unsaturated transferrin help to maintain the concentration of free iron in tissue fluids at levels that are restrictive for uncontrolled microbial growth. Many studies have identified transferrin as one of the major components necessary for the antimicrobial activity of serum [29,30]. Conversely, increased iron stores have been reported to correlate with increased frequency and severity of many bacterial and fungal infections, as well as sepsis [31–34].

In vitro Antimicrobial Effects of Transferrin

These observations have led investigators to consider a biological-based strategy for iron sequestration using exogenous transferrin. Numerous studies have demonstrated the ability of transferrin to restrict microbial growth *in vitro* due to its iron sequestration capacity [35–37]. Pathogenic organisms whose growth is inhibited by transferrin include both Gram-negative and Gram-positive bacterial pathogens such as *Pseudomonas aeruginosa* [38], *Klebsiella pneumonia* [39], *Yersinia pseudotuberculosis* [40], *Acinetobacter baumannii* [8], and *Bacillus anthracis* [41], as well as fungal pathogens, such as *Candida* species and *Histoplasma capsulatum* [42,43].

Our group has also assessed the *in vitro* efficacy of transferrin against diverse bacterial and fungal pathogens. We conducted time-kill curves and determined minimum inhibitory concentrations (MICs) of recombinant human transferrin (rhTransferrin) against *S. aureus* (Gram-positive bacterium), *A. baumannii* (Gram-negative bacterium) and *C. albicans* (fungus). Transferrin had an MIC of 6 μg/ml for the virulent strains *S. aureus* LAC and *A. baumannii* HUMC1, and a 60 μg/ml MIC for virulent *C. albicans* SC5314, demonstrating concentration-dependent static activity against all three pathogens [44]. At the 60 μg/ml concentration (10-fold above the MIC), transferrin mediated a >3 log reduction in *S. aureus* CFUs at 24 hours compared to growth control [44]. For *A. baumannii*, both the 6 and 60 μg/ml concentration mediated 10–100-fold reductions in CFUs/ml at 24 hours compared to growth control. For *C. albicans*, the 60 μg/ml dose mediated minor growth inhibition at 6 hours, and 3-fold reductions in CFUs at 24 hours. Higher concentrations (120 and 360 μg/ml) mediated substantial inhibition of growth at all time points [44].

Because transferrin targets host iron, rather than a biochemical target on microbes, we hypothesized that it would exert minimal selective pressure driving resistance. We found that serial passage of each organism in the presence of a sub-inhibitory concentration of rhTransferrin for 20 generations led to no change in susceptibility. Antimicrobial activity was inhibited by the addition of exogenous iron or iron-loaded siderophores, as well as anti-transferrin antibodies. In addition, intracellular iron levels in all three pathogens were markedly reduced following exposure to rhTransferrin in a dose-dependent manner [44]. Thus, transferrin acts as a static, not cidal, agent against a broad spectrum of human pathogens.

Iron is a critical electron acceptor in the oxidative phosphorylation cascade that leads to ATP generation in both prokaryotes and eukaryotes [45]. Treatment of *A. baumannii*, *S. aureus*, and *C. albicans* with rhTransferrin resulted in disrupted membrane potentials in all

three pathogens in a dose-dependent manner, as early as 1 hour following treatment, with increased effect at 6 hours [44]. Although both *C. albicans* and *S. aureus* experienced some degree of membrane potential recovery at 24 hours, this may have been due to the liberation of intracellular iron stores from dying organisms, which became available to saturate transferrin. Disrupted membrane potentials were maintained when the transferrin was separated by a filter from the microbes, and were totally reversed by the addition of exogenous iron. Thus, the effect of transferrin on microbial membrane potentials appeared to be due to iron sequestration.

Challenges to Standardizing *In vitro* Testing of Transferrin

When testing transferrin MICs to a variety of organisms, we noted substantial variability between assays that used serum from different batches, due to variability in the concentration of iron and iron binding proteins in the serum [44]. Thus, reproducible transferrin MIC testing requires conducting the assay in media without serum. Rich growth media that are normally used for susceptibility testing also posed challenges due to the high levels of free iron. Such media do not recapitulate the normal, exceedingly low free iron levels in human blood and tissues. When tested in RPMI in the absence of serum, human apo-transferrin MICs were highly reproducible. However, the apo-transferrin MICs were substantially lower than the physiological concentration of transferrin (a mixture of apo- and holo-transferrin) in human blood [28]. The amount of apo-transferrin that is required to be added into biological matrices to inhibit microbial growth will be difficult to predict given the complex dynamics of free vs. bound iron in such matrices. Thus, while *in vitro* reproducibility of MIC testing is likely to require assays run in the absence of serum, clinical investigation is going to be required to define how breakpoints set by such assays predict *in vivo* efficacy.

In vivo Validation of Transferrin Efficacy Against Infection

In contrast to the numerous reports on the *in vitro* inhibitory effects of transferrin on microbial growth, there have been fewer investigations into the potential for exogenous transferrin to effectively treat infections *in vivo*. One early study demonstrated decreased mortality rates in mice with experimental candidiasis that were preadministered transferrin [46]. More recently, our group infected mice intravenously with various pathogens to test the ability of rhTransferrin to confer survival against distinct, lethal bloodstream infections. Separate groups of mice were administered *S. aureus*, *A. baumannii*, and *C. albicans*, and treated with human transferrin or placebo. Four doses of 90 and 270 mg/kg/d of rhTransferrin substantially improved survival against all three infections compared to placebo-treated mice [44]. Transferrin treatment significantly reduced tissue bacterial/fungal burden for all three pathogens, as well. Protection mediated by rhTransferrin was reversed by administration of exogenous free iron, or by treatment with transferrin saturated with iron before injection into the mice.

We also sought to determine the potential for transferrin to synergize with antimicrobial therapy, as well as help prevent the emergence of resistance to antimicrobial therapy. We selected rifampin as a representative antibiotic to test against *S. aureus*, because emergence

of bacterial resistance occurs rapidly during rifampin monotherapy due to a single step mutation that results in high level resistance [47]. *In vitro*, a sub-MIC concentration of rhTransferrin (3 μg/ml) synergized with antibiotic treatment, decreasing the *S. aureus* MIC of rifampin from 0.15 μg/ml to 0.019 μg/ml (8-fold decrease) [44]. *In vivo*, when rhTransferrin was combined with rifampin treatment to treat *S. aureus* bacteremia, the emergence of rifampin-resistance escape mutants was markedly reduced [44]. Prevention of resistance could be due to synergy with antibiotics or geometric mass action (antibiotic escape mutant rate * transferrin escape mutant rate = much lower frequency of viable, resistant escape mutants).

Evidence of safety profile from clinical trials

One potential advantage of administering a naturally occurring biologic molecule, as opposed to small molecule chelators, is that supraphysiological levels of transferrin would presumably not alter the normal pathways of iron sequestration and trafficking, but would rather enhance these pathways. Transferrin has already been extensively studied in clinical trials for patients with iron overload, providing important insights into its safety profile [36]. In a series of studies over 10 years ago, stem cell transplant patients undergoing myeloablative chemotherapy were administered intravenous transferrin, in order to counter their high levels of non-transferrin-bound iron (NTBI). A single dose of apo-transferrin (100 mg/kg) was well-tolerated by six stem cell transplant patients following myeloablation, and correlated with a reduction in transferrin saturation, as well as a reduction of NTBI to undetectable levels [48]. Serum samples taken directly after apo-transferrin administration inhibited the growth of *S. epidermidis* [49]. Later studies showed that repeated administration of divided daily doses of 1040 mg/kg total of apo-transferrin were well-tolerated by stem cell transplant patients, with no observable toxicities, and marked decreases in transferrin saturation and unbound iron [50]. An ongoing phase II/III dose escalation study is reported on clinicaltrials.gov [NCT01797055]. Thus, although transferrin is not yet FDA-approved, no substantial toxicity signals have yet been revealed in these trials [36]. Therefore, adjunctive transferrin therapy to treat infections or to reduce resistance emergence may therefore be rapidly translatable.

Conclusions and Future Needs

The impending crisis of antibiotic resistance has created a need for alternative therapies to treat bacterial infections, and strategies that treat the host rather than the bacteria are attractive because they are theoretically less likely to induce resistance. We and others have demonstrated that transferrin has broad, cross-kingdom efficacy both *in vitro* and *in vivo*, and likely represents a superior approach to sequestering iron by using small molecule chelating agents. Multiple clinical trials treating patients with iron overload have already demonstrated acceptable safety profiles, which would thus enable rapid clinical translation of transferrin as a viable antimicrobial therapy. Delivery of supraphysiological levels of recombinant transferrin may also inhibit or slow the development of intrinsic antibiotic resistance. For these reasons, transferrin represents a potentially promising clinical alternative or adjunct to traditional antibiotic treatment, and is worthy of continued study.

Acknowledgments

This work was supported by NIAID R01 AI081719, R56 AI104751, R41 AI106375, R21 AI101750, and R21 AI101492.

Bibliography

1. Schaible UE, Kaufmann SHE. Iron and microbial infection. Nature Reviews Microbiology. 2004; 2:946–953. [PubMed: 15550940]
2. Skaar EP. The Battle for Iron between Bacterial Pathogens and Their Vertebrate Hosts. PLoS Pathog. 2010; 6:e1000949. [PubMed: 20711357]
3. Sutak R, Lesuisse E, Tachezy J, Richardson DR. Crusade for iron: iron uptake in unicellular eukaryotes and its significance for virulence. Trends in Microbiology. 2008; 16:261–268. [PubMed: 18467097]
4. Haley KP, Skaar EP. A battle for iron: host sequestration and Staphylococcus aureus acquisition. Microbes Infect. 2012; 14:217–27. [PubMed: 22123296]
5**. Cassat JE, Skaar EP. Iron in Infection and Immunity. Cell Host & Microbe. 2013; 13:509–519. This comprehensive review describes the role of iron in immunity to various microbial pathogens, and outlines various host and microbe strategies for acquiring and controlling iron. [PubMed: 23684303]
6. Aguila A, Herrera AG, Morrison D, Cosgrove B, Perojo A, Montesinos I, Pérez J, Sierra G, Gemmell CG, Brock JH. Bacteriostatic activity of human lactoferrin against Staphylococcus aureus is a function of its iron-binding properties and is not influenced by antibiotic resistance. FEMS Immunology & Medical Microbiology. 2001; 31:145–152. [PubMed: 11549422]
7. Ibrahim AS, Gebermariam T, Fu Y, Lin L, Husseiny MI, French SW, Schwartz J, Skory CD, Edwards JE, Spellberg BJ. The iron chelator deferasirox protects mice from mucormycosis through iron starvation. The Journal of Clinical Investigation. 2007; 117:2649–2657. [PubMed: 17786247]
8. de De Léséleuc L, Harris G, KuoLee R, Chen W. In Vitro and In Vivo Biological Activities of Iron Chelators and Gallium Nitrate against Acinetobacter baumannii. Antimicrobial Agents and Chemotherapy. 2012; 56:5397–5400. [PubMed: 22825117]
9**. Foley TL, Simeonov A. Targeting iron assimilation to develop new antibacterials. Expert opinion on drug discovery. 2012; 7:831–847. This review discusses recent efforts to develop drugs that prevent bacteria from acquiring iron, including iron chelation approaches, siderophore inhibition, and blocking heme uptake and utilization. [PubMed: 22812521]
10*. Thompson MG, Corey BW, Si Y, Craft DW, Zurawski DV. Antibacterial Activities of Iron Chelators against Common Nosocomial Pathogens. Antimicrobial Agents and Chemotherapy. 2012; 56:5419–5421. This study defined growth inhibiting activity of several newly defined iron chelators against a variety of bacterial pathogens, both Gram-positive and Gram-negative. [PubMed: 22850524]
11. Fischbach MA, Lin H, Liu DR, Walsh CT. How pathogenic bacteria evade mammalian sabotage in the battle for iron. Nature Chemical Biology. 2006; 2:132–138. [PubMed: 16485005]
12**. Caza M, Kronstad JW. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Frontiers in Cellular and Infection Microbiology. 2013; 3 This recent review outlines specific bacterial and fungal strategies to acquire iron in the context of human infection, including receptors and transporters designed to bind iron-sequestering molecules (such as transferrin), and siderophore production and uptake.
13. Miethke M, Marahiel MA. Siderophore-Based Iron Acquisition and Pathogen Control. Microbiology and Molecular Biology Reviews. 2007; 71:413–451. [PubMed: 17804665]
14. Wandersman C, Delepelaire P. Bacterial Iron Sources: From Siderophores to Hemophores. Annual Review of Microbiology. 2004; 58:611–647.
15. Bernier G, Girijavallabhan V, Murray A, Niyaz N, Ding P, Miller MJ, Malouin F. Desketoneoenactin-Siderophore Conjugates for Candida: Evidence of Iron Transport-Dependent Species Selectivity. Antimicrobial Agents and Chemotherapy. 2005; 49:241–248. [PubMed: 15616301]

16. Dorsey CW, Tomaras AP, Connerly PL, Tolmasky ME, Crosa JH, Actis LA. The siderophore-mediated iron acquisition systems of Acinetobacter baumannii ATCC 19606 and Vibrio anguillarum 775 are structurally and functionally related. Microbiology. 2004; 150:3657–3667. [PubMed: 15528653]

17. Funahashi T, Tanabe T, Mihara K, Miyamoto K, Tsujibo H, Yamamoto S. Identification and Characterization of an Outer Membrane Receptor Gene in Acinetobacter baumannii Required for Utilization of Desferricoprogen, Rhodotorulic Acid, and Desferrioxamine B as Xenosiderophores. Biological and Pharmaceutical Bulletin. 2012; 35:753–760. [PubMed: 22687412]

18. Haas H. Molecular genetics of fungal siderophore biosynthesis and uptake: the role of siderophores in iron uptake and storage. Applied Microbiology and Biotechnology. 2003; 62:316–330. [PubMed: 12759789]

19. Heymann P, Gerads M, Schaller M, Dromer F, Winkelmann G, Ernst JF. The Siderophore Iron Transporter of Candida albicans (Sit1p/Arn1p) Mediates Uptake of Ferrichrome-Type Siderophores and Is Required for Epithelial Invasion. Infection and Immunity. 2002; 70:5246–5255. [PubMed: 12183576]

20. Lee J-H, Han Y. Candida albicans can utilize siderophore during candidastasis caused by apotransferrin. Archives of Pharmacal Research. 2006; 29:249–255. [PubMed: 16596999]

21. Noble SM. Candida albicans specializations for iron homeostasis: from commensalism to virulence. Current Opinion in Microbiology. 2013; 16:708–715. [PubMed: 24121029]

22. Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, Aydinok Y, Kattamis A, Kilinc Y, Porter J, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with β-thalassemia. Blood. 2006; 107:3455–3462. [PubMed: 16352812]

23. Vichinsky E. Clinical application of deferasirox: Practical patient management. American Journal of Hematology. 2008; 83:398–402. [PubMed: 18058997]

24. Gordeuk VR, Thuma PE, Brittenham GM, Biemba G, Zulu S, Simwanza G, Kalense P, M'Hango A, Parry D, Poltera AA, et al. Iron Chelation as a Chemotherapeutic Strategy for Falciparum Malaria. The American Journal of Tropical Medicine and Hygiene. 1993; 48:193–197. [PubMed: 8447522]

25. LS, Santos AL, Sodre CS, Valle RA, Silva BA, Abi-chacra EV, Silva LL, Souza-Goncalves AS, Sangenito LS, Goncalves DOP, Souza L, et al. Antimicrobial Action of Chelating Agents: Repercussions on the Microorganism Development, Virulence and Pathogenesis. Current Medicinal Chemistry. 2012; 19:2715–2737. [PubMed: 22455582]

26*. Spellberg B, Ibrahim AS, Chin-Hong PV, Kontoyiannis DP, Morris MI, Perfect JR, Fredricks D, Brass EP. The Deferasirox–AmBisome Therapy for Mucormycosis (DEFEAT Mucor) study: a randomized, double-blinded, placebo-controlled trial. Journal of Antimicrobial Chemotherapy. 2012; 67:715–722. This paper describes a recent randomized, controlled trial of patients with mucormycosis that found that small molecule iron chelation was not safe or effective, highlighting the need for alternate strategies to limit iron availability. [PubMed: 21937481]

27*. Gkouvatsos K, Papanikolaou G, Pantopoulos K. Regulation of iron transport and the role of transferrin. Biochimica et Biophysica Acta (BBA) - General Subjects. 2012; 1820:188–202. This recent review highlights the function of transferrin in iron transport and systemic iron homeostasis. [PubMed: 22085723]

28. Gambino R, Desvarieux E, Orth M, Matan H, Ackattupathil T, Lijoi E, Wimmer C, Bower J, Gunter E. The relation between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. Clinical Chemistry. 1997; 43:2408–2412. [PubMed: 9439462]

29. Sridhar S, Ahluwalia M, Brummer E, Stevens DA. Characterization of an Anticryptococcal Protein Isolated from Human Serum. Infection and Immunity. 2000; 68:3787–3791. [PubMed: 10816550]

30. Watanabe T, Tanaka H, Nakao N, Mikami T, Suzuki M, Matsumoto T. Anti Candida activity of induced transferrin in mice immunized with inactivated Candida albicans. Biological & Pharmaceutical Bulletin. 1997; 20:637–640. [PubMed: 9212981]

31. Hunter RL, Bennett B, Towns M, Vogler WR. Transferrin in disease II: defects in the regulation of transferrin saturation with iron contribute to susceptibility to infection. American Journal of Clinical Pathology. 1984; 81:748–753. [PubMed: 6375346]

32. McDonald CJ, Jones MK, Wallace DF, Summerville L, Nawaratna S, Subramaniam VN. Increased Iron Stores Correlate with Worse Disease Outcomes in a Mouse Model of Schistosomiasis Infection. PLoS ONE. 2010; 5:e9594. [PubMed: 20231891]
33. Sawatzki G, Hoffmann FA, Kubanek B. Acute iron overload in mice: pathogenesis of Salmonella typhimurium infection. Infection and Immunity. 1983; 39:659–665. [PubMed: 6339386]
34. Schaible UE, Collins HL, Priem F, Kaufmann SHE. Correction of the Iron Overload Defect in β-2-Microglobulin Knockout Mice by Lactoferrin Abolishes Their Increased Susceptibility to Tuberculosis. The Journal of Experimental Medicine. 2002; 196:1507–1513. [PubMed: 12461085]
35. Bezkorovainy A. Antimicrobial properties of iron-binding proteins. Advances in Experimental Medicine and Biology. 1981; 135:139–154. [PubMed: 6452038]
36. Brandsma ME, Jevnikar AM, Ma S. Recombinant human transferrin: Beyond iron binding and transport. Biotechnology Advances. 2011; 29:230–238. [PubMed: 21147210]
37. Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Natural resistance, iron and infection: a challenge for clinical medicine. Journal of Medical Microbiology. 2006; 55:251–258. [PubMed: 16476787]
38. Brandsma ME, Diao H, Wang X, Kohalmi SE, Jevnikar AM, Ma S. Plant-derived recombinant human serum transferrin demonstrates multiple functions. Plant Biotechnology Journal. 2010; 8:489–505. [PubMed: 20432512]
39. Lawrence TH, Biggers CJ, Simonton PR. Bacteriostatic inhibition of Klebsiella pneumoniae by three human transferrins. Annals of Human Biology. 1977; 4:281–284. [PubMed: 332055]
40. Salamah AA, al-Obaidi AS. Effect of some physical and chemical factors on the bactericidal activity of human lactoferrin and transferrin against Yersinia pseudotuberculosis. The New Microbiologica. 1995; 18:275–281. [PubMed: 7553362]
41. Rooijakkers SHM, Rasmussen SL, McGillivray SM, Bartnikas TB, Mason AB, Friedlander AM, Nizet V. Human Transferrin Confers Serum Resistance against Bacillus anthracis. Journal of Biological Chemistry. 2010; 285:27609–27613. [PubMed: 20615872]
42. Shiraishi A, Arai T. Antifungal activity of transferrin. Sabouraudia. 1979; 17:79–83. [PubMed: 375440]
43. Sutcliffe MC, Savage AM, Alford RH. Transferrin-Dependent Growth Inhibition of Yeast-Phase Histoplasma capsulatum by Human Serum and Lymph. Journal of Infectious Diseases. 1980; 142:209–219. [PubMed: 7410898]
44**. Lin L, Pantapalangkoor P, Tan B, Bruhn KW, Ho T, Nielsen T, Skaar EP, Zhang Y, Bai R, Wang A, et al. Transferrin Iron Starvation Therapy for Lethal Bacterial and Fungal Infections. Journal of Infectious Diseases. 2014; 210:254–264. This study demonstrated that human transferrin inhibits growth of a broad range of microbes, including Gram-positive (*Staphylococcus aureus*), Gram-negative (*Acinetobacter baumannii*), and fungal (*Candida albicans*) pathogens, by sequestering iron. Intravenously delivered transferrin improves survival of mice challenged with lethal inocula of these pathogens, providing proof of principle that bacterial and fungal infections can be treated *in vivo* with supraphysiologic levels of transferrin to limit iron availability. [PubMed: 24446527]
45. Bird LJ, Bonnefoy V, Newman DK. Bioenergetic challenges of microbial iron metabolisms. Trends in Microbiology. 2011; 19:330–340. [PubMed: 21664821]
46. Abe F, Katoh T, Inaba H, Hotchi M. Experimental candidiasis associated with liver injury. Role of transferrin Mycopathologia. 1988; 104:3–6. [PubMed: 2975353]
47. Spratt BG. Resistance to antibiotics mediated by target alterations. Science. 1994; 264:388–393. [PubMed: 8153626]
48. Sahlstedt L, von Bonsdorff L, Ebeling F, Ruutu T, Parkkinen J. Effective binding of free iron by a single intravenous dose of human apotransferrin in haematological stem cell transplant patients. British Journal of Haematology. 2002; 119:547–553. [PubMed: 12406099]
49. Von Bonsdorff L, Sahlstedt L, Ebeling F, Ruutu T, Parkkinen J. Apotransferrin administration prevents growth of Staphylococcus epidermidis in serum of stem cell transplant patients by binding of free iron. FEMS Immunology \& Medical Microbiology. 2003; 37:45–51. [PubMed: 12770759]
50. Parkkinen J, Sahlstedt L, Von Bonsdorff L, Salo H, Ebeling F, Ruutu T. Effect of repeated apotransferrin administrations on serum iron parameters in patients undergoing myeloablative

conditioning and allogeneic stem cell transplantation. British Journal of Haematology. 2006; 135:228–234. [PubMed: 16925790]

Highlights

- Mammalian transferrin maintains iron homeostasis in serum and extracellular tissue fluid.
- Sequestration of iron is an innate host strategy to prevent pathogenic microbial growth.
- Addition of transferrin limits growth of numerous diverse pathogens in the bloodstream.
- Delivery of exogenous transferrin may offer an attractive antimicrobial approach.