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Iron: an essential nutrient for *Aspergillus fumigatus* and a fulcrum for pathogenesis

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Abstract

Purpose of review—*Aspergillus fumigatus* is a ubiquitous saprophytic fungus that can cause life-threatening invasive aspergillosis in immunocompromised patients. Apart from the immune status of the host only a few characterized virulence factors have been identified. In this review, we describe the role of iron in the manifestation of *A. fumigatus* virulence.

Recent findings—We gathered recent clinical evidence suggesting that tissue iron overload increases the risk of invasive aspergillosis occurrence. Furthermore, we summarize the mechanisms that *A. fumigatus* employs to achieve iron homeostasis and their importance in *A. fumigatus* proliferation in vitro. We describe two recent in-vivo models that clearly demonstrate the importance of iron in *A. fumigatus* growth and invasion.

Summary—Based on these recent findings, therapy aimed at managing *A. fumigatus* iron homeostasis locally could make conditions more favorable to the host.

Keywords

Aspergillus fumigatus; cystic fibrosis; invasive aspergillosis; iron; microbial interaction; pathogenesis

INTRODUCTION

Aspergillus fumigatus is the most prominent fungus in the airways of cystic fibrosis patients [1], and a prominent opportunistic pathogen in neutropenic patients and in patients after lung and liver transplantation [2–6]. *A. fumigatus* biofilms, three-dimensional networks of cellular hyphae and extracellular matrix, are associated with antifungal resistance, resistance to host immunity, and also persistence of infection [7]. With minor exceptions, almost all

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There are no conflicts of interest.

organisms, including *A. fumigatus*, employ iron as a cofactor for fundamental biochemical activities such as oxygen transportation, energy metabolism, and DNA synthesis [8,9]. During infection, the host's innate immune system sequesters iron, decreasing free iron to low levels as a defense mechanism to limit microbial growth [10]. Thus, competition for iron is a critical battleground that determines the outcome of the host–pathogen relationship [11]. Towards this end, pathogenic microbes have developed mechanisms to acquire iron from their hosts [12,13]. In contrast to various bacterial and fungal pathogens, *A. fumigatus* lacks systems for direct uptake of host iron sources and relies on other methods to adjust to the ambient iron concentration [9]. Although *A. fumigatus* response to low iron conditions has been well characterized [9], its adaptations to iron overload are only increasingly being appreciated. The evidence associating *A. fumigatus* virulence with iron is derived from: clinical studies; studies demonstrating the requirement for iron uptake mechanisms to maintain fungal virulence; and animal and in-silico studies that demonstrate a critical role of iron overload to drive fungal growth and invasion. In this review, we highlight recent findings regarding *A. fumigatus* siderophore-mediated iron uptake, intermicrobial competition for iron, the role of iron in lung pathogenesis, and clinical relevance.

CLINICAL EVIDENCE

Increased virulence with iron overload is suggested by the clinical observations of a higher incidence of *Aspergillus* infections in persons with medical diseases that increase tissue iron. The liver plays a key role in iron homeostasis by regulating iron uptake (by producing the iron sequestration protein, hepcidin), providing a key iron store in the body, and by producing ceruloplasmin and transferrin – proteins that are vital for iron metabolism [14]. In persons after liver transplantation, stainable iron in the explanted liver was independently associated with an increased risk of invasive fungal infections [hazard ratio 3.09, 95% confidence interval (CI) 1.45–6.56, $P=0.003$], with *A. fumigatus* infection occurring in 25% of the patients [15]. This increased risk of invasive aspergillosis has also been observed in patients with hematologic malignancies and after autologous and allogeneic hematopoietic cell transplantation (HCT) [16–19]. In these patients, iron overload is likely due to frequent blood transfusions, ineffective erythropoiesis, and defects in iron metabolism [16]. In persons with acute myeloid leukemia and acute lymphoblastic leukemia, invasive fungal infections were associated with higher serum iron, ferritin, and transferrin saturation concentrations than in those persons who did not develop fungal infections [19]. Kontoyiannis et al. [17], in a retrospective study among patients with leukemia and after allogeneic HCT, showed that moderate to severe iron overload, measured histologically in bone marrow iron stores, was associated with an increased risk of IA in a multivariate analysis (hazard ratio 12.3, 95% CI 3.4–44.9, $P<0.0001$). In another study, in patients who died after autologous or allogeneic HCT, there was a significant relationship between hepatic iron overload and invasive aspergillosis [relative risk (RR) 9, 95% CI 1.6–50.3, $P=0.012$] [16]. Similar findings have been described by Ozyilmaz *et al.* [18] who showed in persons after HCT that serum ferritin concentration (>1000 ng/ml) increased the risk of fungal pneumonia, the majority due to *Aspergillus* sp. (62%) [odds ratio (OR) 3.42, 95% CI 1.06–11.42, $P=0.045$].

RELEVANCE OF IRON FOR *ASPERGILLUS FUMIGATUS* IN VITRO

Iron sequestration, either pharmaceutically by use of iron chelators [20], or by natural substances, for example, the *Pseudomonas aeruginosa* siderophore pyoverdine [21■■■], or the *P. aeruginosa* phage Pf4 [22■■■], have been shown to be detrimental for the *A. fumigatus*. *P. aeruginosa* and *A. fumigatus* frequently co-exist in the same micro-environment, for example, in lungs of cystic fibrosis, lung transplant, or neutropenic patients [2,5,6,23]. Because iron is their common essential nutrient, both micro-organisms have developed measures to secure iron for their own needs. *A. fumigatus* produces four hydroxamate-containing siderophores: ferricrocin and hydroxyferricrocin (HFC) for intracellular iron trafficking, and also fusarinine C (FsC) and its derivative triacetylfulsarinine C (TAFC) for extracellular iron scavenging (Fig. 1) [24–26]. Critical for the biosynthesis of those four hydroxamate-containing siderophores is the enzyme ornithine mono-oxygenase, termed SidA [9,26], catalyzing oxygen and NADPH-dependent hydroxylation of L-ornithine to N5-L-hydroxyornithine. An *A. fumigatus* lacking SidA (Af *sidA*) was unable to establish invasive aspergillosis in a mouse model [24,27]. *P. aeruginosa* metabolites have been shown to inhibit [28,29] and induce apoptosis [30■] in *A. fumigatus*. In situations of chronic *P. aeruginosa* resistance in vivo, such as in cystic fibrosis lungs, surviving *P. aeruginosa* phenotypes are more powerful inhibitors of *A. fumigatus* than non-CF *P. aeruginosa* isolates [31]. We recently showed, via studies with 24 deletion mutants, that the most important *P. aeruginosa* molecule responsible for antifungal activity is the siderophore pyoverdine, and that the mechanism of antifungal action is sequestration of ferric iron [21■■■]. Because pyoverdine cannot be used as a xenosiderophore for *A. fumigatus*, the fungus suffers from iron shortage, and increases expression of genes needed for production of its own siderophores, for example, *sidA*, *hapX*, and *mirB*, while saving energy by down-regulating genes expressed during times of iron-abundancy, for example, *sreA*, *cccA*, *cycA*, and *acoA* (as summarized in Fig. 1) [21■■■]. Recently, MirC also has been shown to play an important role in bio-synthesis of the *A. fumigatus* intracellular siderophore ferricrocin, and to contribute to the maintenance of iron homeostasis [32]. Antifungal activity of pyoverdine seems to be exclusively based on iron denial to the fungus, because experiments with suitable mutants revealed no contribution of the pyoverdine downstream effectors ExoA or PrpL protease [21■■■]. Effects of pyoverdine on *A. fumigatus* are independent of the bacterial strain producing the pyoverdine, the type of pyoverdine (I, II, or III), or the *A. fumigatus* strain used for experiments [21■■■,33].

Normal siderophore production, and hence sufficient iron assimilation, is of high importance for *A. fumigatus*. It has recently been shown that pharmacological interference with SidA production, and hence hydroxamate siderophore production, reduced fungal growth *in vitro* [34■].

RELEVANCE OF IRON FOR *ASPERGILLUS FUMIGATUS* IN VIVO

The critical role of *A. fumigatus* genes involved in iron acquisition, as outlined above, have been examined in vivo using animal infection models [35–37]. In addition to the in-vitro studies, the correlation of iron and *A. fumigatus* virulence was predicted by a computational model that showed that iron acquisition was essential for the development of invasive

aspergillosis [38■■■]. However, to date, few animal studies have directly examined the association between iron overload and fungal virulence. Here, we describe a fungal keratitis and a *A. fumigatus* tracheal transplantation model that clearly suggests the importance of iron in *A. fumigatus* growth and invasion.

FUNGAL KERATITIS MODEL

Aspergillus fumigatus and other filamentous fungi are major causes of fungal keratitis in developing nations, accounting for 1 million cases annually in Asia and Africa [39]. As treatments for these infections are often limited, Leal *et al.* examined the role of targeting iron acquisition as a novel treatment strategy for these infections. The first series of experiments examined whether iron availability would regulate *A. fumigatus* growth. Mice (C57BL/6) were treated with iron dextran or deferoxamine (DFO), an iron chelator, which *A. fumigatus* can use as a xenosiderophore in acquiring iron. Mice were then infected by direct corneal stromal injection with *A. fumigatus* conidia. In these experiments, the mice treated with iron dextran and DFO demonstrated higher fungal mass at 48 h after inoculation compared to control mice, vehicle-treated animals. Conversely, the addition of topical lactoferrin, an iron-chelating host protein, lessened the fungal mass and colony-forming unit (CFU) per eye compared to mice treated with vehicle control. Together, these studies demonstrate that fungal growth in the cornea is determined by bioavailable iron [38■■■]. The next series of experiments examined the role of fungal siderophores, using *A. fumigatus* iron acquisition mutants. In these experiments, researchers examined the role of *A. fumigatus* mutants in the production of intracellular and extracellular siderophores. Figure 1 depicts the pathways involved in fungal siderophore production. In these studies, mutants of *sidA*, *sidF*, *sidD*, *sidI*, and *sidH* had significantly lower fungal burden and CFU than wild-type or their respective reconstituted mutants. Whereas mutants of intracellular siderophores, including *sidC* and *sidG* or *ftrA*, a mutant that lacks a membrane-bound iron transport channel, making it deficient in the uptake of extra-cellular iron, were not significantly different than wild-type in the corneal infection model. Moreover, treatment of *A. fumigatus* with an HMG-CoA reductase inhibitor (statin class of medications), which inhibits mevalonate production, a critical step in siderophore biosynthesis (see Fig. 1), and deferi-prone (iron chelator that cannot be used by *A. fumigatus* as a xenosiderophore) inhibited *A. fumigatus* growth in the corneal infection model. Together, these data are consistent with the siderophore data mentioned previously, suggesting that extracellular iron siderophores are essential for *A. fumigatus* virulence, and that blocking iron uptake or reducing bioavailable iron attenuates *A. fumigatus* growth.

ORTHOTOPIC TRACHEAL TRANSPLANT MODEL OF ASPERGILLUS FUMIGATUS INVASION

One in three lung transplant recipients suffer from an *Aspergillus*-related pulmonary disease that presents as chronic colonization, airway anastomotic infections, or invasive aspergillosis [40–42]. Other than immune suppression, the factors that determine disease presentation are unknown [43,44]. To address this question, our research group developed a murine orthotopic tracheal transplant (OTT) model of *A. fumigatus* infection to study the

transplanted host–pathogen interaction. In these studies, we performed both allogeneic (BALB/c donor transplanted into a C57BL/6 recipient) and syngeneic (C57BL/6 donor and recipient) to study the effects of alloimmune-mediated rejection on *A. fumigatus* transplant invasion, as measured histologically. In our initial studies, we found that *Aspergillus* became more deeply invasive as the allograft underwent progressive rejection-mediated ischemia [45]. Subsequently, we showed that up-regulating endothelial hypoxia-inducible factor (HIF)-1 α (a stimulator of angiogenesis and vascular repair) limited the invasion of the mould [46]. Together, these findings suggested that alloimmune-mediated microvascular damage precipitated a more virulent *A. fumigatus* phenotype, and that by preventing microvascular damage, through HIF-1 α up-regulation, we were able to attenuate this virulence. To further elucidate these findings, we examined the consequences of microvascular damage in the OTT model. We found that damage resulted in diffuse microhemorrhage, which increased the tissue iron content, as measured histologically and by inductively coupled-mass spectroscopy. We then conducted a series of gain and loss-of-function experiments. In these experiments, the depth of *A. fumigatus* invasion progressively increased in: allografts beginning at day 8 post-transplantation, the onset of iron accumulation in the graft; iron rich tracheal transplants from mice with hemochromatosis, a genetic disorder that increases tissue iron content; allograft recipients treated with DFO; and syngrafts treated with a topical solution containing iron sulfate [47]. However, the depth of invasion decreased in allograft recipients treated with the iron chelator, deferasirox, which is not useful to *A. fumigatus* as a xenosiderophore [47]. We then posited that *A. fumigatus* mutants with severe iron intolerance would be less invasive in the OTT model during high allograft iron conditions. These mutants included: *hapX*, which lacks *hapX* – a gene that is required for adaptation to iron starvation through repression of iron-dependent pathways [36]; *sreA*, which lacks the *sreA* gene encoding a repressor of iron uptake during high iron conditions [48]; and the double knockout iron intolerant mutant strain *sreA/cccA*, which lacks both *sreA* and *cccA* – a gene that is critical for *A. fumigatus* iron detoxification [49]. The wild-type strain (AF77, similar genetic background as the mutants) was studied concurrently and served as the control. In iron-overload conditions, the iron-intolerant mutant (*sreA/cccA*) was significantly ($P < 0.05$) less invasive than both the wild-type and the *sreA* moulds. The data indicate that knockout of both *sreA* and *cccA* in *A. fumigatus* was required to lessen fungal invasion. Taken together, these data indicate that iron is a critical determinant of *A. fumigatus* allograft invasion and that this virulence can be mitigated by reducing tissue iron content.

CONCLUSION

The understanding of the role of iron in *A. fumigatus* pathogenicity has advanced enormously during the past decade. Iron is the central nutrient for *A. fumigatus*, and interference with iron availability is detrimental for the fungus. Inhibition of *A. fumigatus* siderophores has been shown to affect the fungal growth both *in vitro* and *in vivo*. The models described here suggest that iron availability is crucial not only for *A. fumigatus* proliferation but also plays a pivotal role in the fungal virulence. In addition, there is an increase in the clinical evidence, suggesting that immunocompromised patients with iron-overload after transplantation are at high risk of developing invasive aspergillosis. Having a

better understanding of the role of iron in *A. fumigatus* pathogenicity will facilitate the development of treatment strategies and novel diagnostics for invasive aspergillosis. Inhibiting *A. fumigatus* siderophore synthesis pharmacologically, or use of certain chelators locally, could favor the host.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■ of outstanding interest

1. Sabino R, Ferreira JA, Moss RB, et al. Molecular epidemiology of *Aspergillus* collected from cystic fibrosis patients. *J Cyst Fibros* 2015; 14:474–481. [PubMed: 25459562]
2. Walsh T, Stevens D. Chapter 347: aspergillosis In: Goldman L, Schafer A, editors. *Cecil Medicine*, 24th ed. New York: Elsevier; 2011.
3. Qin J, Fang Y, Dong Y, et al. Radiological and clinical findings of 25 patients with invasive pulmonary aspergillosis: retrospective analysis of 2150 liver transplantation cases. *Br J Radiol* 2016; 89:e429–e435.
4. Pedersen M, Seetharam A. Infections after orthotopic liver transplantation. *J Clin Exp Hepatol* 2014; 4:347–360. [PubMed: 25755581]
5. Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. *Thorax* 2015; 70:270. [PubMed: 25354514]
6. Gregg KS, Kauffman CA. Invasive aspergillosis: epidemiology, clinical aspects, and treatment. *Semin Respir Crit Care Med* 2015; 36:662–672. [PubMed: 26398533]
7. Reichhardt C, Ferreira JA, Joubert LM, et al. Analysis of the *Aspergillus fumigatus* biofilm extracellular matrix by solid-state nuclear magnetic resonance spectroscopy. *Eukaryot Cell* 2015; 14:1064–1072. [PubMed: 26163318]
8. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. *Biochem J* 2011; 434(Pt 3):365–381. [PubMed: 21348856]
9. Haas H Iron: a key nexus in the virulence of *Aspergillus fumigatus*. *Front Microbiol* 2012; 3:28. [PubMed: 22347220]
10. Cassat JE, Skaar EP. Iron in infection and immunity. *Cell Host Microbe* 2013; 13:509–519. [PubMed: 23684303]
11. Marx JJM. Iron and infection: competition between host and microbes for a precious element. *Best Pract Res Clin Haematol* 2002; 15:411–426. [PubMed: 12401315]
12. Tong Y, Guo M. Bacterial heme-transport proteins and their heme-coordination modes. *Arch Biochem Biophys* 2009; 481:1–15. [PubMed: 18977196]
13. Caza M, Kronstad JW. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. *Front Cell Infect Microbiol* 2013; 3:80. [PubMed: 24312900]

14. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. *J Res Med Sci* 2014; 19:164–174. [PubMed: 24778671]
15. Alexander J, Limaye AP, Ko CW, et al. Association of hepatic iron overload with invasive fungal infection in liver transplant recipients. *Liver Transpl* 2006; 12:1799–1804. [PubMed: 16741903]
16. Altes A, Remacha AF, Sarda P, et al. Frequent severe liver iron overload after stem cell transplantation and its possible association with invasive aspergillosis. *Bone Marrow Transplant* 2004; 34:505–509. [PubMed: 15286693]
17. Kontoyiannis DP, Chamilos G, Lewis RE, et al. Increased bone marrow iron stores is an independent risk factor for invasive aspergillosis in patients with high-risk hematologic malignancies and recipients of allogeneic hematopoietic stem cell transplantation. *Cancer* 2007; 110:1303–1306. [PubMed: 17614303]
18. Ozyilmaz E, Aydogdu M, Sucak G, et al. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload. *Bone Marrow Transplant* 2010; 45:1528–1533. [PubMed: 20118991]
19. Iglesias-Osma C, Gonzalez-Villaron L, San Miguel JF, et al. Iron metabolism and fungal infections in patients with haematological malignancies. *J Clin Pathol* 1995; (48):223–225. [PubMed: 7730481]
20. Nazik H, Penner JC, Ferreira JA, et al. Effects of iron chelators on the formation and development of *Aspergillus fumigatus* biofilm. *Antimicrob Agents Chemother* 2015; 59:6514–6520.
21. Sass G, Nazik H, Penner J, et al. Studies of *Pseudomonas aeruginosa* mutants indicate pyoverdine as the central factor in inhibition of *Aspergillus fumigatus* biofilm. *J Bacteriol* 2018; 200. This study concerns intermicrobial interactions of *A. fumigatus* and *P. aeruginosa*. Pyoverdine was identified as the most potent *P. aeruginosa* siderophore that is capable of inhibiting *A. fumigatus* growth by iron starvation.
22. Secor PR, Sass G, Nazik H, Stevens DA. Effect of acute predation with bacteriophage on intermicrobial aggression by *Pseudomonas aeruginosa*. *PLoS One* 2017; 12:e0179659. [PubMed: 28622385] This study highlights the impact of *P. aeruginosa* infection by a species of bacteriophages on the intermicrobial interactions with *A. fumigatus* in cystic fibrosis.
23. de Bentzmann S, Plesiat P. The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. *Environ Microbiol* 2011; 13:1655–1665. [PubMed: 21450006]
24. Schrettl M, Bignell E, Kragl C, et al. Distinct roles for intra- and extracellular siderophores during *Aspergillus fumigatus* infection. *PLoS Pathog* 2007; 3:1195–1207. [PubMed: 17845073]
25. Haas H Fungal siderophore metabolism with a focus on *Aspergillus fumigatus*. *Nat Prod Rep* 2014; 31:1266–1276. [PubMed: 25140791]
26. Blatzer M, Schrettl M, Sarg B, et al. SidL, an *Aspergillus fumigatus* transacetylase involved in biosynthesis of the siderophores ferricrocin and hydro-xyferricrocin. *Appl Environ Microbiol* 2011; 77:4959–4966. [PubMed: 21622789]
27. Hissen AHT, Chow JM, Pinto LJ, et al. Survival of *Aspergillus fumigatus* in serum involves removal of iron from transferrin: the role of siderophores. *Infect Immun* 2004; 72:1402–1408. [PubMed: 14977945]
28. Mangan A Interactions between some aural *Aspergillus* species and bacteria. *J Gen Microbiol* 1969; 58:261–266. [PubMed: 4982824]
29. Blyth W, Forey A. The influence of respiratory bacteria and their biochemical fractions on *Aspergillus fumigatus*. *Med Mycol* 1971; 9:273–282.
30. Shirazi F, Ferreira JA, Stevens DA, et al. Biofilm filtrates of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients inhibit preformed *Aspergillus fumigatus* biofilms via apoptosis. *PLoS One* 2016; 11:e0150155. [PubMed: 26930399] This article confirms the antagonistic interaction between *A. fumigatus* and *P. aeruginosa* in cystic fibrosis. It also provides evidence that metabolites of *P. aeruginosa* can initiate *A. fumigatus* programmed cell death.
31. Ferreira JA, Penner JC, Moss RB, et al. Inhibition of *Aspergillus fumigatus* and its biofilm by *Pseudomonas aeruginosa* is dependent on the source, phenotype and growth conditions of the bacterium. *PLoS One* 2015; 10:e0134692. [PubMed: 26252384]

32. Mulvihill ED, Moloney NM, Owens RA, et al. Functional investigation of iron-responsive microsomal proteins, including MirC, in *Aspergillus fumigatus*. *Front Microbiol* 2017; 8:418. [PubMed: 28367141]
33. Nazik H, Moss RB, Karna V, et al. Are cystic fibrosis *Aspergillus fumigatus* isolates different? Intermicrobial interactions with *Pseudomonas*. *Mycopathologia* 2017; 182:315–318. [PubMed: 27822731]
34. Martin Del Campo JS, Vogelaar N, Tolani K, et al. Inhibition of the flavin-dependent monooxygenase siderophore A (SidA) blocks siderophore bio-synthesis and *Aspergillus fumigatus* growth. *ACS Chem Biol* 2016; 11:3035–3042. [PubMed: 27588426] This article developed a novel screening assay to test selective targeted small molecules that can inhibit siderophore biosynthesis in *A. fumigatus*.
35. Schrettl M, Bignell E, Kragl C, et al. Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence. *J Exp Med* 2004; 200:1213–1219. [PubMed: 15504822]
36. Schrettl M, Beckmann N, Varga J, et al. HapX-mediated adaption to iron starvation is crucial for virulence of *Aspergillus fumigatus*. *PLoS Pathog* 2010; 6:e1001124. [PubMed: 20941352]
37. Goetting V, Lee KA, Woods L, et al. Inflammatory marker profiles in an avian experimental model of aspergillosis. *Med Mycol* 2013; 51:696–703. [PubMed: 23651177]
38. Oremland M, Michels KR, Bettina AM, et al. A computational model of invasive aspergillosis in the lung and the role of iron. *BMC Syst Biol* 2016; 10:34. [PubMed: 27098278] This is the first in-silico model of invasive aspergillosis. This model validates the importance of iron levels in for *A. fumigatus* growth and virulence during invasive pulmonary aspergillosis.
39. Leal SM Jr, Roy S, Vareechon C, et al. Targeting iron acquisition blocks infection with the fungal pathogens *Aspergillus fumigatus* and *Fusarium oxysporum*. *PLoS Pathog* 2013; 9:e1003436. [PubMed: 23853581]
40. Hsu JL, Ruoss SJ, Bower ND, et al. Diagnosing invasive fungal disease in critically ill patients. *Crit Rev Microbiol* 2011; 37:277–312. [PubMed: 21749278]
41. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. *Medicine (Baltimore)* 1999; 78:123–138. [PubMed: 10195093]
42. Patterson JE. Epidemiology of fungal infections in solid organ transplant patients. *Transpl Infect Dis* 1999; 1:229–236. [PubMed: 11428994]
43. Abad A, Fernández-Molina JV, Bikandi J, et al. What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. *Rev Iberoam Micol* 2010; 27:155–182. [PubMed: 20974273]
44. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin Microbiol Rev* 2009; 22:447–465. [PubMed: 19597008]
45. Hsu JL, Khan MA, Sobel RA, et al. *Aspergillus fumigatus* invasion increases with progressive airway ischemia. *PLoS One* 2013; 8:e77136. [PubMed: 24155924]
46. Jiang X, Hsu JL, Tian W, et al. Tie2-dependent VHL knockdown promotes airway microvascular regeneration and attenuates invasive growth of *Aspergillus fumigatus*. *J Mol Med (Berl)* 2013; 91:1081–1093. [PubMed: 23797537]
47. Hsu JL, Manouvakova OV, Clemons KV, et al. Microhemorrhage-associated tissue iron enhances the risk for *Aspergillus fumigatus* invasion in a mouse model of airway transplantation. *Sci Transl Med* 2018; (10):429. This is the first study that demonstrates an association between *A. fumigatus* virulence with tissue iron overload as a result of allo-immune mediated vessel destruction in a mouse model of tracheal transplantation.
48. Schrettl M, Kim HS, Eisendle M, et al. SreA-mediated iron regulation in *Aspergillus fumigatus*. *Mol Microbiol* 2008; 70:27–43. [PubMed: 18721228]
49. Gsaller F, Eisendle M, Lechner BE, et al. The interplay between vacuolar and siderophore-mediated iron storage in *Aspergillus fumigatus*. *Metallomics* 2012; 4:1262–1270. [PubMed: 23151814]

KEY POINTS

- Interference with iron availability is detrimental for *A. fumigatus*.
- Loss of siderophores inhibits *A. fumigatus* proliferation.
- Iron availability is crucial for *A. fumigatus* invasiveness *in vivo*.
- Immunocompromised patients with iron overload post transplantation are at high risk of developing invasive aspergillosis.

FIGURE 1.

Schematic representation of *Aspergillus fumigatus* siderophore biosynthesis and iron regulation. Fe: iron, FusC: fusarinine C, HMG-CoA: 3-hydroxy-3methylglutaryl-coenzyme, TAFC: triacetyl fusarinine C, SidA: ornithine monooxygenase, SidC: ferricrocin nonribosomal peptide synthetase (FC NRPS), SidF: Transacylase, SidD: Fusarinine C nonribosomal peptide synthetase (FSC NRPS), SidG: Transacetylase, SidI: Mevalonyl-CoA ligase, SidH: Mevalonyl-CoA hydratase, RIA: reductive iron assimilation enzyme complex (involves iron reduction by metalloreductases such as FreB and uptake by a protein complex consisting of FetC and FtrA), EstB: TafC specific esterase, MirB: TAFC specific importer, CccA: iron importer to vacuole, HapX: bZip CCAAT-binding transcription factor, SreA: GATA transcription factor, FtrA: high-affinity iron permease, AmcA: putative mitochondrial ornithine transporter, FreB: iron reductase, FetC: ferroxidase, SidJ: fusarinine C esterase. Not shown: The HapX-mediated inhibition of iron-consumption in iron-dependent pathways other than CccA. The high iron activating function of HapX on iron-dependent pathways other than CccA.