



Published in final edited form as:

*J Trauma Acute Care Surg.* 2017 March ; 82(3): 557–565. doi:10.1097/TA.0000000000001338.

## Modeling *Acinetobacter baumannii* wound infections: The critical role of iron

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### Abstract

**Background**—*Acinetobacter baumannii* has emerged as an increasingly important and successful opportunistic human pathogen due to its ability to withstand harsh environmental conditions, its characteristic virulence factors and quick adaptability to stress.

**Methods**—We developed a clinically relevant murine model of *A. baumannii* traumatic wound infection to determine the effect of local wound environment on *A. baumannii* virulence. Mice underwent rectus muscle crush injury combined with ischemia created by epigastric vessel ligation, followed by *A. baumannii* inoculation. Reiterative experiments were performed using 1) a mutant deficient in the production of the siderophore acinetobactin, or 2) iron supplementation of the wound milieu. Mice were euthanized 7 days later and rectus muscle analyzed for signs of clinical infection, HIF1 $\alpha$  accumulation, bacterial abundance and colony morphotype. To determine the effect of wound milieu on bacterial virulence, the *Galleria mellonella* infection model was utilized.

**Results**—The combination of rectus muscle injury with ischemia and *A. baumannii* inoculation resulted in 100% incidence of clinical wound infection that was significantly higher compared to other groups (n=15/group, p<0.0001). The highest level of wound infection was accompanied with the highest level of *A. baumannii* colonization (p<0.0001) and the highest degree of HIF1 $\alpha$  accumulation (p<0.05). *A. baumannii* strains isolated from injured/ischemic muscle with clinical infection displayed a rough morphotype and a higher degree of virulence as judged by *G. mellonella* killing assay as compared to smooth morphotype colonies isolated from injured muscle

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The data was partially presented at the 34th Surgical Infection Society Meeting Baltimore, Maryland, May 1–3, 2014. It has not been published elsewhere.

Conflict of interest statement: No conflicts are declared.

**Author contributions:** IF performed study design and experiments; MK analyzed and interpreted the data, performed a literature search, and wrote the draft of the manuscript; NB performed experiments, analyzed and interpreted the data, participated in the experimental design; AZ, JD, LC performed experiments and analyzed the data; LA analyzed the data and critically revised the manuscript; OZ and JA designed and analyzed the experiments and wrote the manuscript.

without clinical infection (100% vs. 60%, n=30 Log-Rank test, p=0.0422). Iron supplementation prevented wound infection (n=30, p<0.0001) and decreased HIF1 $\alpha$  (p=0.039643). Similar results of decrease in wound infection and HIF1 $\alpha$  were obtained when *A. baumannii* wild type was replaced with its derivative mutant BasD deficient in acinetobactin production.

**Conclusions**—The ability of *A. baumannii* to cause infections in traumatized wound relies on its ability to scavenge iron and can be prevented by iron supplementation to the wound milieu.

## Keywords

*Acinetobacter baumannii*; BasD; wound injury; ischemia; infection; iron; HIF1 $\alpha$

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## Background

Over the past thirty years, *Acinetobacter baumannii* has gained recognition as an increasingly important opportunistic human pathogen. Worldwide, an increasing number and severity of *A. baumannii* outbreaks have occurred in various settings, such as hospitals, long-term care facilities, military bases, and within the civilian community (1–4). *A. baumannii*'s ability to persist in harsh environmental conditions coupled with its alarmingly rapid rate of antibiotic resistance acquisition places this pathogen as one of the six most problematic multi-drug resistant (MDR) healthcare-associated pathogens worldwide (5). Although *A. baumannii* is known for its ability to cause a broad range of infectious complications, from skin and soft tissue infections to ventilator-associated pneumonia and catheter-related blood stream infections (6), here we focus on *A. baumannii* wound infections as they relate to tissue trauma and host stress.

*A. baumannii* traumatic wound infections have become a topic of recent interest with reports of increasing incidences of outbreaks among victims of combat injuries and natural disasters (7). Recent data from Iraq and Afghanistan identify highly resistant strains of *A. baumannii* to be some of the most common organisms causing severe and often lethal wound infections (4, 8, 9). Trauma related *A. baumannii* burn wound infections and osteomyelitis are also emerging as commonly encountered problems in the injured military personnel (10–12). In the nonmilitary setting, *A. baumannii* has been reported to be a cause of surgical site infections in some institutions (13, 14) and an infrequent cause of skin and soft tissue infections in the intensive care unit setting (1). Especially troubling are the antimicrobial resistance profiles of *A. baumannii* wound infections, with reports of epidemics caused by multidrug-resistant strains as well as strains resistant to all available antimicrobials (15).

The increasing success of *A. baumannii* as a human pathogen lies in its virulence factors as well as its quick adaptability to changing environmental conditions (16). The virulence factors responsible for the long-term survival and wide-spread transmissibility in the health care environment include the *A. baumannii*'s ability to attach to and persist on solid and otherwise abiotic surfaces in the form of a biofilm as well as its ability to resist desiccation and disinfection (17, 18). *A. baumannii* is significantly less permeable to biocidal substances, harbors efflux pumps that facilitate its persistence on nosocomial surfaces, and thrives in the presence of ethanol (19). *A. baumannii* has been shown to easily spread from infected patients to the environment through the aerosol route and can survive in the

nosocomial environment for up to 13 days (20). Importantly, *A. baumannii* is rarely found outside of the healthcare setting and exhibits a low human carrier rate (20). To illustrate this point, *A. baumannii* has been recovered from all military treatment facilities sampled on the aeromedical evacuation route from Iraq and Afghanistan; however, *A. baumannii* was found on the skin of only 1 of 160 screened patients and in 1 of 49 soil samples (genetically separate from the clinical isolates), suggesting that its reservoir of infection persists within treatment facilities but rarely beyond (3). These characteristics coupled with the ability of *A. baumannii* to use horizontal gene transfer to acquire multi-drug resistance, make this a particularly problematic and virulent pathogen.

*A. baumannii* has developed additional virulence factors that allow it to evade host defenses. One important innate host immune response is the sequestration of essential metal nutrients such as iron away from pathogens, termed nutritional immunity (21, 22). *A. baumannii* has developed a variety of high-affinity iron acquisition systems to overcome nutritional immunity and scavenge iron away from the host (22). Production of the acinetobactin siderophore is one such mechanism (23). Iron-limited conditions upregulate *A. baumannii* genes involved in iron acquisition as well as genes involved in growth, motility and respiration (24). Deletion and insertion inactivation of genes coding for acinetobactin synthesis and transport impairs *A. baumannii* virulence and reduces its lethality when tested with the *Galleria mellonella* and the mouse sepsis models (23). We therefore hypothesize that iron depletion plays a significant role in *A. baumannii* traumatic wound infection pathogenesis and posit that iron abundance in the wound milieu prevents *in-vivo* virulence activation and protects the host from wound infection.

In order to inform molecular mechanisms responsible for host morbidity and mortality due to *A. baumannii* wound infections, there is a growing need to develop clinically relevant animal models (25). The aim of this study was to establish a reproducible murine model of *A. baumannii* wound infection. We demonstrate that *A. baumannii* wound infection success is dependent on the degree of tissue trauma, with the requirement of tissue injury for abscess development and increased virulence with addition of tissue ischemia. We also show that iron abundance in the wound milieu prevents *in-vivo* virulence activation and protects the host from wound infection, suggesting a key role of iron in *A. baumannii* infection pathogenesis. Our findings inform potential targets for treatment and prevention strategies against this highly problematic pathogen.

## Methods

### Bacteria

*A. baumannii* ATCC 19606<sup>T</sup> and its derivative mutant *basD* (26), deficient in the production of siderophore acinetobactin, were used in the experiments. *A. baumannii* was plated on tryptic soy agar (TSA) from a frozen stock and grown overnight at 37°C. A few colonies were then suspended in liquid TSB medium, sub-grown for 1 hour on a heated shaker and then adjusted to an OD<sub>600</sub> of 0.5. Bacterial suspension was centrifuged at 3,300 × g for 10 min, the excess TSB removed and the remaining pellet was re-suspended in the same volume of 0.9% normal saline. The resulting microbial suspension was used for wound inoculation.

### Mouse model of *Acinetobacter baumannii* wound infection

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Chicago (IACUC protocol 72090). Seven- to nine-week old male C57B/L6 mice were used for all experiments. Mice were routinely fed tap water and Harland Teklad feed (Madison, WI) under 12-h light/dark cycles. All animals were allowed to acclimate for at least 48 hours before surgery. Knowing that tissue trauma related to combat wounds, such as blast injuries and gunshot wounds, is often associated with vascular injury leading to tissue ischemia and necrosis, we designed a more clinically relevant model incorporating both, injury and ischemia. A vertical skin incision was made under sterile conditions and the rectus abdominis muscle was exposed by undermining the skin. Muscle injury was performed using forceps to lightly crush the rectus muscle. Ischemia was induced by double suture ligation of the rectus muscle superiorly and inferiorly with care taken to include the epigastric vascular bundle in the suture ligature. The wound was then inoculated with 100  $\mu$ L of *A. baumannii* suspension ( $10^6$  CFU/mL) in 0.9% normal saline for 2 minutes, followed by washout with 0.9% normal saline and skin closure. Mice were randomly assigned into four groups (n=15/group): 1. *A. baumannii* (AB) inoculation without muscle injury or ischemia (sham + AB), 2. Muscle injury alone followed by *A. baumannii* inoculation (Injury + AB), 3. Muscle ischemia only followed by *A. baumannii* inoculation (Isch + AB), 4. Muscle injury and ischemia followed by *A. baumannii* inoculation (Injury/Isch + AB). Mice were euthanized on post-operative day 7 (POD7) and wounds were examined for the presence of gross abscesses. The rectus muscle was excised, homogenized in 10% glycerol and cultured on MacConkey agar. Rectus muscle tissues were assayed for HIF1- $\alpha$  expression as described below.

### Role of iron in *A. baumannii* wound infection

To assess the role of iron in *A. baumannii* wound infection, reiterative experiments were performed using the above model. Following *A. baumannii* inoculation, 200  $\mu$ L of 100 mM FeCl<sub>3</sub> (Sigma Aldrich) in 0.9% normal saline was used as a washout solution prior to skin closure in place of 0.9% normal saline alone (n=30/group). Mice were euthanized on POD7 and wounds were examined for the presence of gross abscesses. The rectus muscle was excised, homogenized in 10% glycerol and cultured on MacConkey agar. Rectus muscle tissues were also used in HIF1- $\alpha$  assays as described below.

### HIF1 $\alpha$ assay

HIF1 $\alpha$  tissue levels were assayed by western blot analysis. Resected rectus muscle tissues were weighed, homogenized in 10% glycerol using a sterile glass tissue grinder (Corning), snap frozen in liquid nitrogen and kept in  $-80^{\circ}\text{C}$ . Ice-cold lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% Triton X-100, and phenylmethylsulfonyl fluoride, 0.1 mM (Sigma)] was added to the homogenized tissues at the ratio of 10:1 and incubated for 15 min on ice. Following centrifugation at  $9,300 \times g$  for 15 minutes, the supernatants were boiled for 5 min with Laemmli sample buffer (Novex), electrophoresed through 10% SDS-polyacrylamide gels, and then transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore). The membranes were blocked for 1 hour in TBS Tween-20 buffer (ThermoScientific) with 4% nonfat dry milk (LabScientific). Membranes were then

incubated with primary anti-HIF-1 $\alpha$  antibody (Novus Biologicals), followed by the corresponding horseradish peroxidase-coupled secondary antibody (at dilutions recommended by the vendors). Beta-actin was used as an internal housekeeping protein control. The membranes were developed using ECL Western Blotting Detection Reagents (GE Healthcare). Densitometry analyses were performed using ImageJ software.

### A. *baumannii* virulence assay using *Galleria mellonella*

Last-instar (VII) larvae of the wax moth *G. mellonella* in the weight range of 250–350 mg were stored in the dark and used within 1 week of shipment (Best Bet, Inc., Blackduck, MN). Intrahaemocoelic injection of 10  $\mu$ l of *A. baumannii* suspension diluted to OD<sub>600</sub> of 0.2 was performed using the KDS-100 single syringe pump (KD Scientific). Larvae were placed between the thumb and forefinger and needle was carefully placed in the cuticle. *A. baumannii* isolates were recovered from sham + AB muscle samples from mice that did not display gross abscess formation but were nonetheless culture positive, as well as and from Injury/Isch + AB muscle samples with gross abscess formation. Isolates were grown overnight on MacConkey agar and subgrown for 1 hour in liquid TSB media. The suspension was then diluted to OD<sub>600</sub> of 0.2 in 0.9% normal saline and 10  $\mu$ l injected into the larvae to determine differences in larvae mortality and hence virulence among the tested strains. After each injection, the larva was carefully removed away from the needle and placed in a 5-cm petri dish. Fifteen larvae were used per group in two replicates. The larvae were then incubated at 37°C in the dark and followed for mortality over the next 72 hours. Dead larvae were recognized by lack of movement and color change to black.

### Statistics

Statistical analysis was performed using GraphPad Prism 7.00 (GraphPad Software, La Jolla, California). Categorical variables were compared using chi-square test, and continuous variables using Student's t-test. Non-parametric statistical analysis with Bonferroni correction for multiple comparisons was used for the infection analysis in mice. *Galleria mellonella* survival curves were created using Kaplan-Meier method with log-rank test. Statistical significance was established at  $P < 0.05$ .

## Results

### Muscle injury and ischemia are synergistic and sufficient for *A. baumannii* wound infection in mice

Gross infection determined by presence of an abscess was noted in 100% of mice on POD7 following rectus muscles injury and ischemia with AB inoculation (Injury/Isch + AB) (Fig. 1A). None of the control groups without *A. baumannii* inoculation (i.e. ischemia alone, injury alone, or ischemia and injury) developed clinically detectable infections. Concurrently, we confirmed 0% rate of abscess formation in the sham + AB group, 0% abscess formation in the Isch + AB group and 20 % rate of abscess formation in the Injury + AB group, this was however not a statistically significant difference (n=15 per group,  $p=0.68$ ; Bonferroni corrected  $\alpha'=0.0024$ ) (Fig. 1B). These findings significantly differed from the 100% rate of abscess formation in the Injury/Isch + AB group (n=15 per group,  $*p<0.0001$ ; Bonferroni corrected  $\alpha'=0.0024$ ) (Fig. 1B).

### ***A. baumannii* densely colonizes injured ischemic muscle tissues**

Upon culture of homogenized rectus muscle wound tissues at the time of mice sacrifice at POD7, wounds subjected to muscle injury, ischemia and *A. baumannii* inoculation (Injury/Isch + AB) displayed  $10^{10}$  average colony forming unit (CFU) count per milligram of tissue that was significantly higher compared to the other groups (n=30 tissue samples/group, \*p<0.0001) (Fig. 1C). Only few tissue samples in Injury + AB and Isch + AB groups were colonized by *A. baumannii* at the average  $10^6$  CFU/mg tissue, these two groups did not significantly differ from one another (n=30, p=0.776).

### **HIF1 $\alpha$ expression is greatest in injured and ischemic tissues infected by *A. baumannii***

The groups of mice with at least one insult (Injury alone or Ischemia alone) had elevated levels of HIF1 $\alpha$  compared to rectus muscle of non-treated mice (sham alone) (p=0.018348 and 0.049987, respectively) (Fig. 2). *A. baumannii* (AB) inoculation led to an increase in HIF1 $\alpha$  in all groups (Sham + AB vs Sham alone, p=0.0076; Injury + AB vs Injury alone, p=0.031782; and Isch + AB vs Ischemia alone, p=0.045198), highlighting the role of *A. baumannii* (AB) in HIF1 $\alpha$  expression. The triple insult consisting of injury, ischemia and *A. baumannii* (Injury/Isch + AB) led to ~3 fold increase in HIF1 $\alpha$  production compared to Isch + AB (p=0.045954) and Injury + AB (p=0.035776) (Fig. 2).

### **Iron plays a critical role in the pathogenesis of *A. baumannii* wound infection**

Since iron is a cofactor of HIF1 $\alpha$  prolyl hydroxylase required for HIF1 $\alpha$  ubiquitination (Fig. 3), we next hypothesized that the high level of HIF1 $\alpha$  accumulation in the tissue of the Injury/Isch + AB animal group may be related, in part, to *A. baumannii*'s ability to locally scavenge iron from the wound milieu away from host tissues. We therefore introduced iron into the wound milieu of our murine model of *A. baumannii* wound infection. The wounds washed out with 200  $\mu$ L of 100 mM FeCl<sub>3</sub> solution following muscle injury, ischemia and *A. baumannii* inoculation, did not develop wound infections (n=30 per group, \*p<0.0001) (Fig. 4). Reiterative experiments were performed using an *A. baumannii* ATCC 19606<sup>T</sup>

BasD mutant deficient in the synthesis of the iron scavenging siderophore acinetobactin and hence without ability to sequester iron from the local environment. Results indicated that following muscle injury, ischemia and BasD *A. baumannii* inoculation, none of 30 animals developed wound infections (Fig. 4).

### **Injured and ischemic tissues exposed to *A. baumannii* produce lower levels of HIF1 $\alpha$ in the presence of iron**

We next evaluated the role of iron in the accumulation of HIF1 $\alpha$  in wound tissues using Injury/isch + AB groups of mice. The presence of *A. baumannii* significantly increased HIF1 $\alpha$  expression (p=0.043862). However, wounds supplemented with iron contained decreased levels of HIF1 $\alpha$  (p=0.039643) (Fig. 5). Similarly, HIF1 $\alpha$  level was decreased when WT strain of *A. baumannii* was replaced by BasD deficient in iron scavenging (injury/Isch/WT vs injury/Isch/ BasD, p=0.044488).

### **A. baumannii isolates from injured ischemic tissues display a more virulent phenotype**

We have noted that *A. baumannii* shifted its colony morphotype to a rough-edge appearance and larger colonies when cultured directly from abscesses of injured and ischemic muscle (Fig. 6A). To determine whether this change in morphotype correlates with enhanced virulence, we used a *G. mellonella* infection model, an established invertebrate animal model already used to study the virulence of different pathogens including *A. baumannii* (23, 27). Seventy-two hours after intrahaemocoelic injection, rough colony morphotypes caused significantly higher mortality rates compared to the smooth colony morphotype (100% vs. 60% respectively, n=30, Log-Rank test, p=0.0422) (Fig. 6B). The morphotype change was not stable in culture reverting back to the smooth morphotype following several subcultures in nutrient-rich TSA medium.

## **Discussion**

While the epidemiology of *A. baumannii* wound infections has been extensively studied, their pathogenicity remains poorly understood. In order to elucidate the molecular mechanisms responsible for *A. baumannii* wound infections, we established a reproducible and clinically relevant murine model of traumatic *A. baumannii* wound infection. We provide evidence that the pathogenesis of *A. baumannii* wound infections is dependent on the degree of tissue trauma and the composition of the wound milieu. We have previously shown that the wound environment plays a significant contributory role in infectious pathogenesis using a *Pseudomonas aeruginosa* wound infection model in which rectus muscle injury providing contact with fascia was sufficient for *P. aeruginosa* to cause wound infection through activation of its iron scavenging systems (28). However, as our current work demonstrated, a more severe insult combining muscle injury with ischemia was required for *A. baumannii* wound infection in mice. This difference may reflect a higher capacity of *P. aeruginosa* to trigger its virulence in response to multiple host derived signals (28–34), and hence explain, in part, its reputation as a highly virulent pathogen. Without the local microenvironmental cues present in a wound complicated by injury and ischemia, *A. baumannii* may not be sufficiently activated to cause a clinical wound infection.

*A. baumannii*'s most potent virulence activation system relies on its ability to respond to iron limitation through iron chelation (35, 36). We therefore hypothesized that the ability of *A. baumannii* to cause traumatic wound infection in Injury/Ischemia mouse model relies on its ability to scavenge iron from the wound milieu. We chose the combination of injury and ischemia to model *A. baumannii* wound infections as it reflects the clinical soft-tissue infections associated with war trauma in which *A. baumannii* is the lead pathogen (4, 37). It is well known that ischemic tissues of traumatized wounds are characterized by overexpression and stabilization of a master transcriptional regulator HIF1 $\alpha$  (38). HIF1 $\alpha$  expression is linked to iron metabolism where it senses and regulates iron content in tissues (39). It is a compensatory mechanism aimed at increasing the oxygen content of tissues through the activation of various genes encoding proteins such as transferrin and ferritin (39). Ferritin-mediated iron sequestration can stabilize HIF1 $\alpha$  by inhibiting its hydroxylation (Fig. 3) (40). It can also induce bacteria to activate their iron sequestration systems (i.e., siderophores) that compete for iron with ferritin (21, 41, 42). These

interactions can lower tissue-associated iron levels and induce further accumulation of HIF1 $\alpha$  in an iterative loop of signal exchange. Our data clearly show evidence for a synergistic effect of injury, ischemia and *A. baumannii* exposure on the development of traumatic *A. baumannii* wound infection related to increased HIF1 $\alpha$  production. These intersecting systems between tissue trauma compensatory mechanisms and bacterial virulence expression are dampened by the presence of iron in the wound milieu as competition for iron is eliminated. While it may seem counterintuitive that supplementation of iron, a known growth factor for bacteria, actually prevented wound infection in this model, the interrelationship between iron scavenging by *A. baumannii* and the role of iron in post-translational HIF1 $\alpha$  stabilization and accumulation can play a yet-unrecognized role. Results from the present study suggest that iron excess could promote the ubiquitination of HIF1 $\alpha$  and shut down the expression of bacterial virulence factors.

While HIF1 $\alpha$  is an essential mediator of the cellular response network to hypoxia, its overexpression has been shown to associate with poor clinical outcomes (43–45). Additionally, pathogenic bacteria have been shown to recognize HIF1 $\alpha$  and respond to its accumulation with enhanced virulence (29) and to further enhance HIF1 $\alpha$  production in host tissues (46, 47). As such, the accumulation of HIF1 $\alpha$  in our model may serve as a quorum sensing signal for *A. baumannii*, enhancing its virulence and leading to wound infection. However, whether excess accumulation of HIF1 $\alpha$  in injured and ischemic tissues infected by *A. baumannii* is just a biomarker of oxygen and iron deficiency or whether it is directly responsible for *A. baumannii* success at causing an infection, requires further clarification.

There is clearly a complex molecular dialogue that occurs between host tissues and invading pathogens that, dependent on local environmental cues and resources, shifts the clinical trajectory from either pathogen clearance to gross infection. This can be seen in the group of animals with non-traumatized wounds that failed to develop clinical infection despite contamination with significant inoculum of *A. baumannii*, as well as in the ability of the wound environment to select for the two reported morphotypes, rough and smooth, that significantly vary in virulence. The context-dependency of this response clearly involves a complex interrelationship between iron and iron-scavenging mechanisms of both the invading pathogen and the host tissues. The use of high resolution multi-omic analyses will facilitate the identification and understanding of the bacterial factors involved in this process.

In summary, here we present a novel model of *A. baumannii* wound infection that demonstrates the critical role of the wound environment and bacterial functions in the pathogenesis of these critical infections and the emergence of morphotypes with enhanced virulence. Manipulation of iron within traumatic wounds at risk for *A. baumannii* infection could represent a novel anti-virulence approach to prevent this lethal infection.

## Acknowledgments

This work was supported by NIH grant 5R01 GM062344 and DDRCC grant P30DK42086.

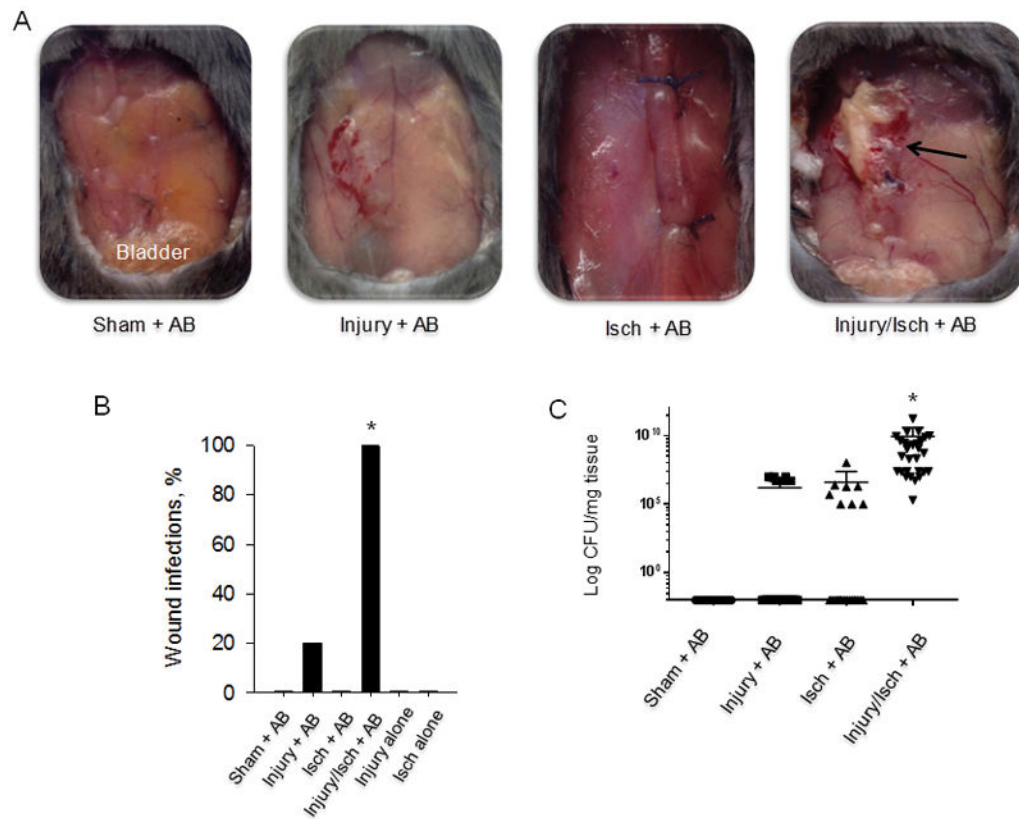


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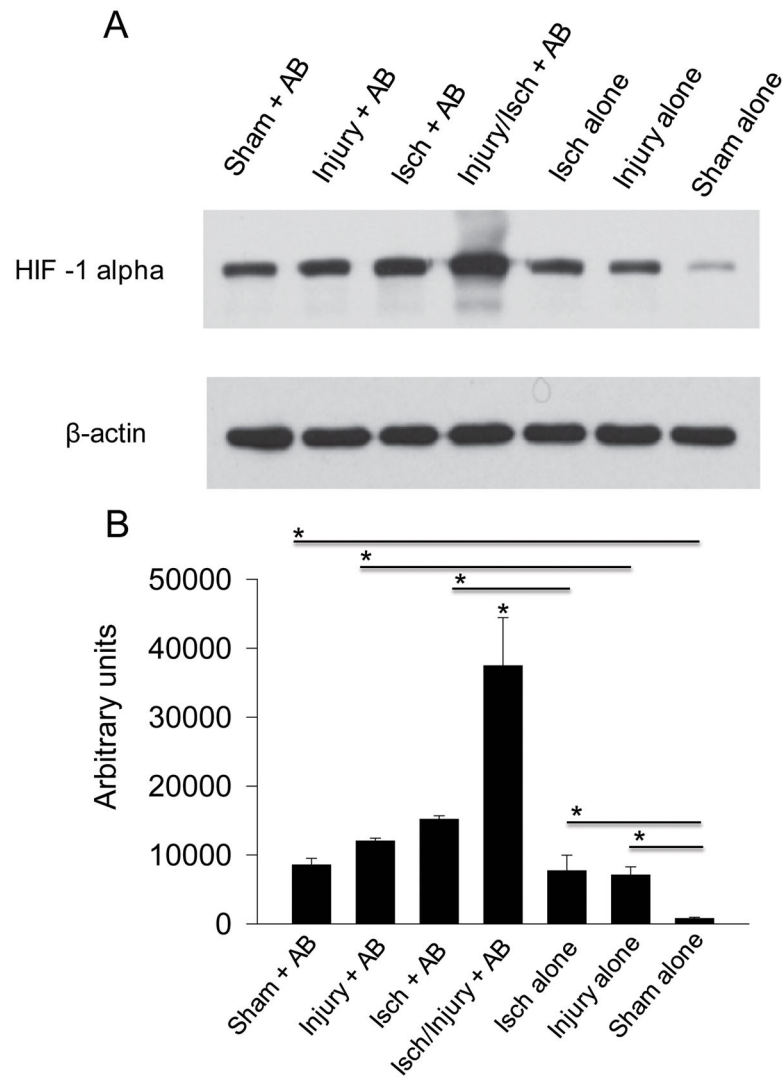
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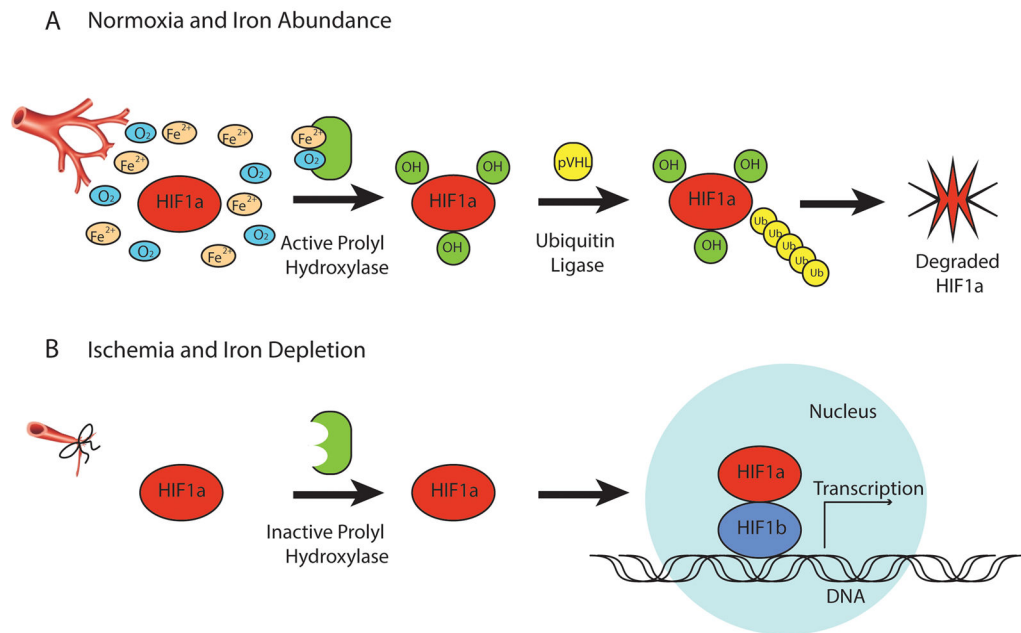


**Figure 1. Muscle injury and ischemia are required for *Acinetobacter baumannii* wound infection in mice**

(A), Images of wounds on post-operative day 7 (POD7) in four treatment groups of mice. Black arrow denotes an abscess. (B), Percent of animals with gross wound infection in each group (n=15/group). The rate of abscess formation in Injury + AB group reached 20% but was not significantly higher compared to Sham + AB group (P=0.68; Bonferroni corrected  $\alpha'$ =0.0024). The rate of abscess formation in the Injury/Isch + AB group was significantly greater than in all other groups ( $p < 0.0001$ ; Bonferroni corrected  $\alpha'$ =0.0024) (C), Bacterial density of *A. baumannii* in mouse tissues. All injured and ischemic tissue samples (Inj/Isch + AB) displayed significantly greater *A. baumannii* colonization compared to other groups (n=30; 15 mice/group  $\times$  2 tissue samples each, \* $p < 0.0001$ , Mann-Whitney test). A negligible level of contamination (seen as black dots on the  $\times$  axis) was found in majority of samples in other groups. Each dot on graph represents CFU value of each tissue sample. AB (*A. baumannii*), Injury (muscle injury), Isch (muscle ischemia).

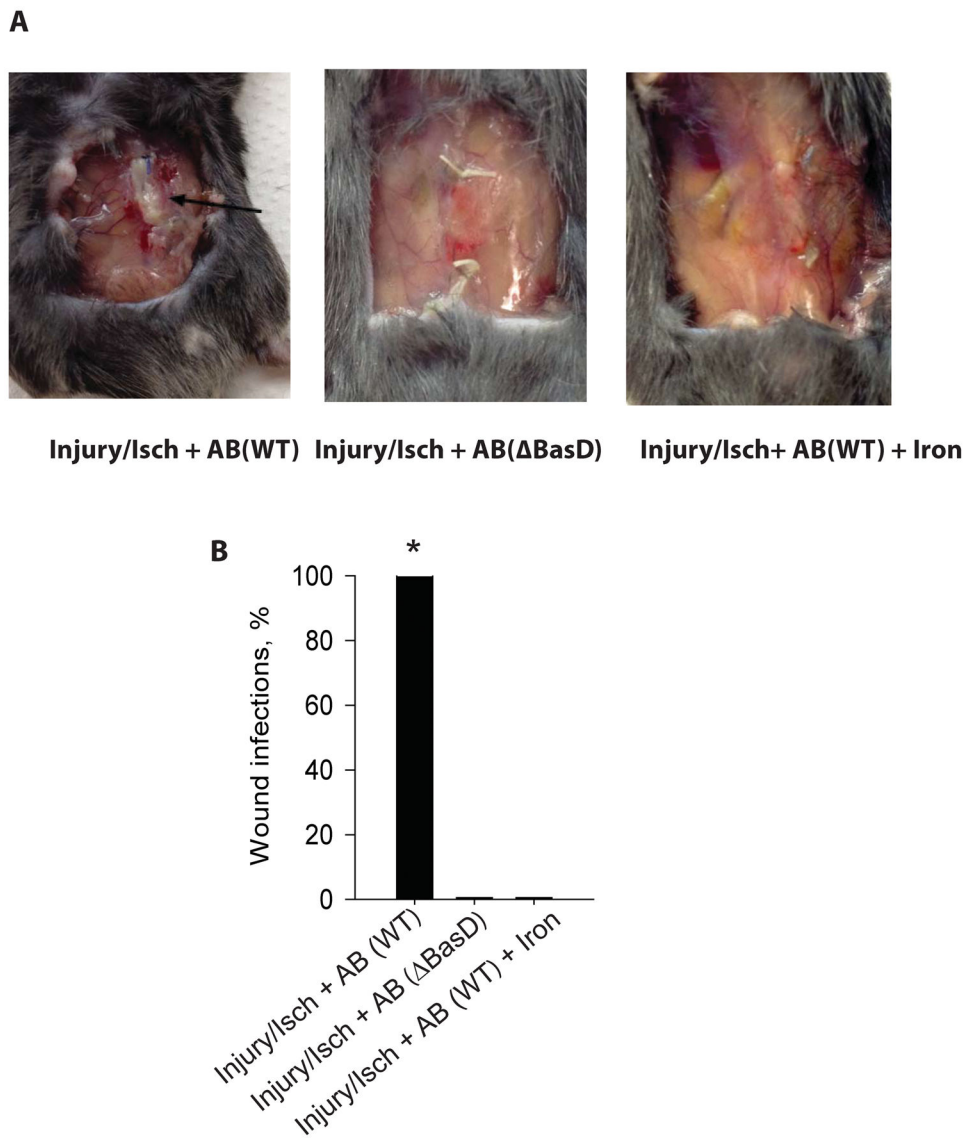


**Figure 2. HIF1 $\alpha$  is most abundant in injured ischemic tissues exposed to *A. baumannii*** (A), Western blot analysis of muscle tissues isolated on POD7. (B), Protein band densities evaluated by ImageJ (n=3, \* $p$ <0.05). AB (*A. baumannii*), Injury (muscle injury), Isch (muscle ischemia). Error bars indicate standard deviation.



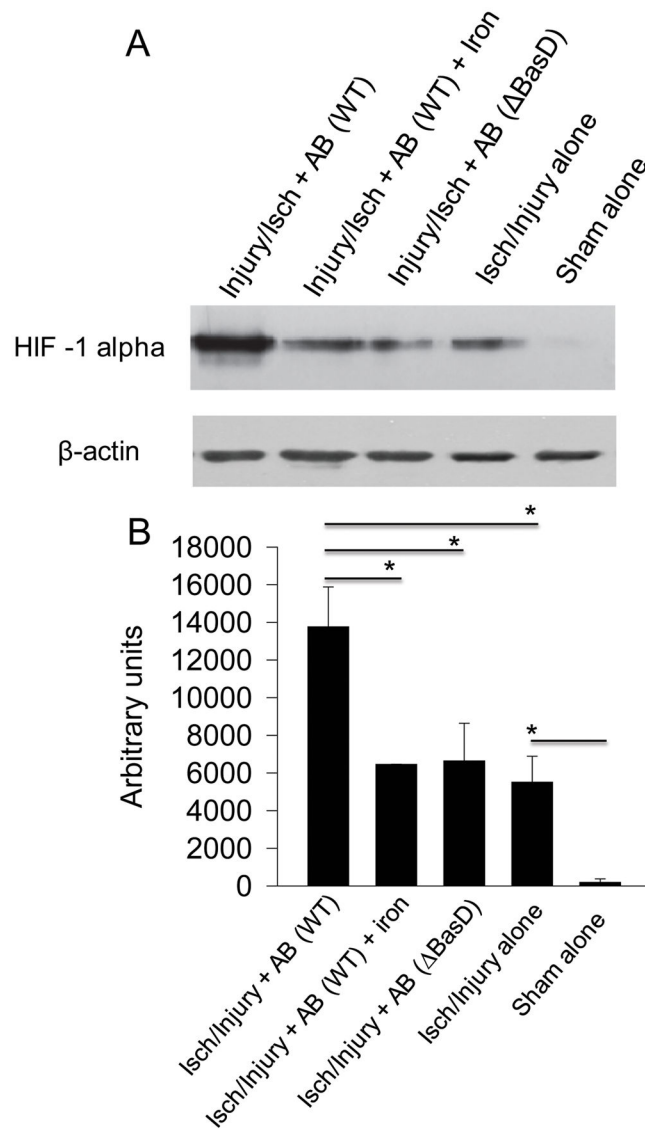
**Figure 3. The role of oxygen and iron in HIF1 $\alpha$  degradation**

(A), In the presence of normoxia and iron abundance, HIF1 $\alpha$  undergoes hydroxylation by active form of prolyl hydroxylase, which requires oxygen and iron cofactors. This is followed by proteasomal degradation of HIF1 $\alpha$  mediated by ubiquitin ligase. (B), Limitation of oxygen and iron, characteristic of injured and ischemic tissues, are essential for post-translational HIF1 $\alpha$  stabilization and accumulation, affecting gene transcription.



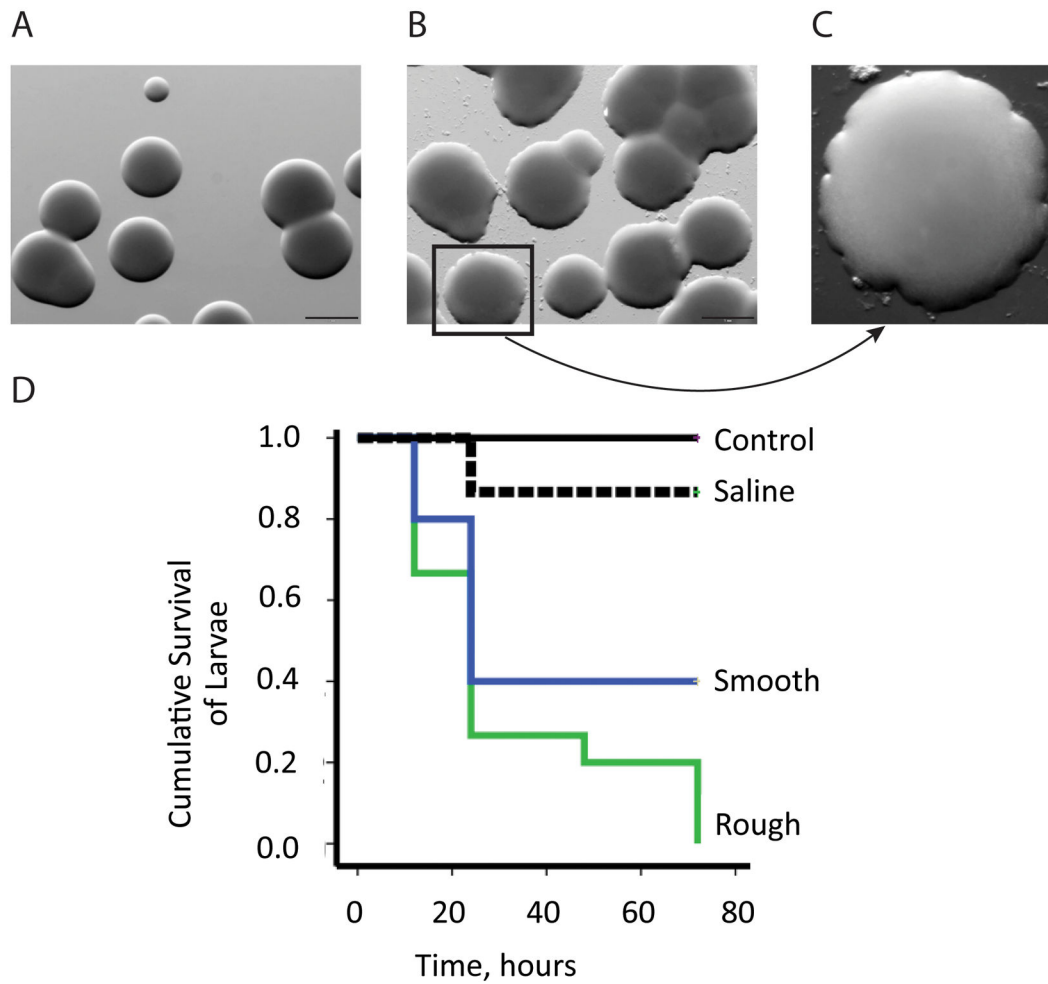
**Figure 4. Iron plays a critical role in *A. baumannii* wound infection**

(A), Images of wounds on POD7 in three treatment groups of mice. Black arrow denotes an abscess. (B), Percent of animals with gross wound infection in each group (n=30/group, \*p<0.0001). AB (*A. baumannii*), Injury (muscle injury), Isch (muscle ischemia), WT (wild type), BasD (*A. baumannii* mutant deficient in scavenging iron).



**Figure 5. Abundance of iron reduces HIF1 $\alpha$  in injured ischemic tissues exposed to *A. baumannii*** **(A)**, Western blot analysis of muscle tissues isolated on POD7. **(B)**, Protein band densities evaluated by ImageJ (n=3, \* $p$ <0.05). AB (*A. baumannii*), Injury (muscle injury), Isch (muscle ischemia), WT (wild type), BasD (*A. baumannii* mutant). Error bars indicate standard deviation.





**Figure 6. *A. baumannii* isolates from injured ischemic tissues have higher virulence phenotype (A), Smooth *A. baumannii* morphotype colonies isolated from abscess-negative wounds (1mm size bar). (B), Rough *A. baumannii* morphotype isolated from abscess-positive wounds (1mm size bar). (C) Magnification of a rough colony. (D), Kaplan-Meier survival curves of *G. mellonella* injected with smooth and rough morphotype colonies. Control larvae were not injected with any solution. Saline group larvae served as negative control in which the injection of 10  $\mu$ l of sterile saline solution was performed (n=30,  $P < 0.05$  comparing mortality induced by smooth vs. rough morphotypes).**