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Original article

Modulation of virulence gene expression in *Staphylococcus aureus* by interleukin-1β: Novel implications in bacterial pathogenesis

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Abstract

The effect of IL-1β on *Staphylococcus aureus* was investigated in terms of mRNA expression profile of bicomponent leukotoxins (Luk ED, Luk PV, HlgA, and HlgCB) as well as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Upon exposure to higher concentrations of IL-1β, *S. aureus* expressed significantly higher levels of MSCRAMMs mRNA {fibronectin-binding protein (FnBp), fibrinogen-binding protein or clumping factor (Clf), and collagen-binding protein (Cna)} and had significantly lower expression of mRNAs for bicomponent leukotoxins. Sequential in vitro passing of *S. aureus* in the absence of rhIL-1β resulted in reduced binding to rhIL-1β resulted in lack of significant changes in virulence gene expression upon exposure to low or high concentrations of rhIL-1β. It is possible that IL-1β modulates the pathogenic potential of *S. aureus* by altering its virulence gene expression to adapt to the host's inflammatory micromilieu. The ability to express higher levels of MSCRAMMs and low levels of leukotoxins might contribute towards the successful invasion and persistence of *S. aureus* in chronic inflammatory conditions. Determination of the mechanisms of IL-1-induced alterations in *S. aureus* gene expression may lead to the identification of novel therapeutic targets against this ever-evolving opportunistic pathogen.

Keywords: *S. aureus*; Leukotoxins; MSCRAMMs; Inflammation; IL-1; Virulence genes; IL-1 binding; Pathogenicity island; Microarray; Two component signal transduction

1. Introduction

Staphylococcus aureus can persist as a commensal in the body, or cause wide ranging diseases [1]. It can also invade a variety of mammalian cells [2,3]. Previous studies have shown various means by which *S. aureus* overcomes the host's innate immune response [4]. Voyich et al., using a transcriptome analysis, demonstrated the upregulation of genes involved in virulence, oxidative stress, and genes coding for hypothetical proteins in *S. aureus* that survived in the intracellular milieu of neutrophils [5]. Neutrophils express a variety of inflammatory mediators including IL-1. We have previously shown that *S. aureus* binds to IL-1β and that its replication is enhanced in the presence of IL-1β and selected synthetic peptides derived from IL-1β [6]. A very recent study has shown that IL-1β enhances growth in *S. aureus* biofilms [7]. Boelens et al. have described enhanced susceptibility to subcutaneous abscess formation and persistent infection with *Staphylococcus epidermidis* around catheters where IL-1β is present [8].

Bacteria can sense environmental factors and express relevant virulence genes [9]. The identification and understanding

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of the function of specific genes that are expressed in the bacterium under the influence of the host micromilieu merits further investigation [10]. In this study, we examined the effect of recombinant human IL-1β (rhIL-1β) on the virulence genes {bicomponent leukotoxins and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)} expression in *S. aureus*. *S. aureus* grown in the presence of higher concentration of IL-1β had significantly higher levels of MSCRAMMs mRNAs while the mRNA levels of bicomponent leukotoxins were significantly reduced compared to respective controls.

2. Materials and methods

2.1. Bacteria

Fresh isolates of *S. aureus* from patients admitted to UT-Bowld Hospital were obtained from UT-HSC Clinical Microbiological Laboratories without any identification pertaining to the patients. A blood culture isolate from a patient with invasive staphylococcal disease was used in the current investigation on gene expression. For receptor cross-linking, strains isolated from blood as well as bronchoalveolar lavage fluid (BAL) of de-identified patients were used. In addition, another fresh BAL isolate of *S. aureus* was passed in vitro six times to artificially attenuate the strain. The parent fresh isolate of this strain was stored at −80 °C from the primary subculture to compare gene expression with its in vitro passed strain.

2.2. Affinity cross-linking of ''IL-1 receptor'' on S. aureus

S. aureus cultures grown to logarithmic phase in DMEM were washed in binding buffer twice. The bacterial cells {5×10^7 colony forming unit (CFU)} were then resuspended in 100 μl of binding buffer containing protease inhibitor cocktail mix for bacterial cells (Sigma, St. Louis, MO) and 10 nM of ^{125}I-labeled rhIL-1β {without and with 1000-fold excess of unlabeled recombinant human IL-1β (rhIL-1β; R&D Systems, Minneapolis, MN)}. This mixture was incubated at 4 °C for 4 h for affinity binding. The bacterial pellets were then washed twice in binding buffer and resuspended in 100 μl of binding medium containing protease inhibitor cocktail. One hundred micrograms of the cross-linking agent disuccinimidyl suberate (DSS; Pierce Chemical Co., Rockville, IL) was added to the mixture and incubated at 4 °C for 2 h. The cells were washed twice, lysed and the lysates were analyzed on a 12.5% gel under denaturing reducing conditions. The gel was dried and exposed to Kodak Scientific Imaging film for 18 h.

2.3. Specific IL-1β binding to fresh and in vitro passed S. aureus

Competitive radio-ligand-binding assay was used to detect IL-1β binding to the surface of *S. aureus*. Approximately, 1×10^5 CFU of *S. aureus* was inoculated into 5.0 ml of DMEM containing 25 mM HEPES buffer and 10 mM L-Glutamine and incubated at 37 °C for 3 h. Aliquots of cultures were taken and incubated with 100,000 cpm (0.02 μCi) of ^{125}I-labeled rhIL-1β. For the determination of non-specific binding, non-radioactive rhIL-1β (0, 1 ng, 10 ng, 100 ng and 1 μg) was added and incubated at 15 °C for 3 h together with ^{125}I-labeled rhIL-1β. The bacteria were then centrifuged and the pellets were washed three times in cold DMEM. The radioactivity in the pellet was counted for 10 min using gamma counter. Specific binding was calculated by subtracting non-specific binding from total binding. The experiment was repeated three times with each strain (fresh, three times and six times passed isolates).

2.4. Estimation of mRNA levels of leukotoxins and MSCRAMMs in S. aureus grown in medium with and without rhIL-1β

For initial gene expression studies, 1×10^6 CFU of fresh blood culture isolates of *S. aureus* obtained from logarithmic phase of growth was added to 10 ml of L-broth containing 0, 5 (low) and 25 ng/ml (high) rhIL-1β. Another fresh isolate of *S. aureus* obtained from BAL of a de-identified patient admitted with acute respiratory distress syndrome (ARDS) was passed in vitro six times and 1×10^6 CFU of bacteria from the sixth passage obtained from the logarithmic phase of growth was inoculated into 10 ml of L-broth containing 0, 5 (low) and 25 ng/ml (high) rhIL-1β. The cultures were incubated for 12 h and the bacterial pellets were collected by centrifugation. The *S. aureus* pellet was treated with 10 μg/ml lysostaphin (Sigma) and total RNA was isolated using TRI-Reagent-Chloroform (Sigma) extraction. Approximately 4–5 μg of *S. aureus* RNA was reverse transcribed. The mRNA levels of *S. aureus* DNA gyrase (GYR), bicomponent leukotoxins (Luk ED, Luk PV, HIgA, HIgCB) and MSCRAMMs {fibronectin-binding protein (FnBp), fibrinogen-binding protein/clumping factor (Clf A), and collagen-binding protein (Cna)} were quantified by real-time RT-PCR using Sybrgreen protocol (ABI, CA, ABI Prism Model 7900) (Table 1). We determined the specificity of the products by melting/dissociation curve. The ratios of the Ct values of GYR to that of the specific messages described above were calculated and the reciprocals of the Ct values were used for plotting the graphs. The experiments were repeated at least three times and were run in triplicates at each time.

2.5. cDNA microarray for comprehensive monitoring of the relative mRNA abundance of S. aureus sequences

Total RNA was isolated from *S. aureus* grown in L-broth containing 0 or 25 ng/ml rhIL-1β. The RNA integrity was determined by capillary electrophoresis using RNA 6000 Nano Lab-on-a-Chip kit and the Bioanalyzer 2100 (Agilent Technologies). First strand cDNA was synthesized from 10 μg to 15 μg of total RNA using SuperScript II Double-Stranded cDNA Synthesis kit (Invitrogen) and Random Hexamer Primers (Promega, Madison WI). DNase I (Promega)-Fragmented cDNAs (~5 μg) were labeled with biotinylated dd-UTP using