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Detection of the host immune response to *Burkholderia mallei* heat-shock proteins GroEL and DnaK in a glanders patient and infected mice☆

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Abstract

We examined, by enzyme-linked immunosorbent assay and Western blot analysis, the host immune response to 2 heat-shock proteins (hsps) in a patient and mice previously infected with *Burkholderia mallei*. The patient was the first reported human glanders case in 50 years in the United States. The expression of the *groEL* and *dnaK* operons appeared to be dependent upon a σ^{32} RNA polymerase as suggested by conserved heat-shock promoter sequences, and the *groESL* operon may be negatively regulated by a controlling invert repeat of chaperone expression (CIRCE) site. In the antisera, the GroEL protein was found to be more immunoreactive than the DnaK protein in both a human patient and mice previously infected with *B. mallei*. Examination of the supernatant of a growing culture of *B. mallei* showed that more GroEL protein than DnaK protein was released from the cell. This may occur similarly within an infected host causing an elevated host immune response to the *B. mallei* hsps.
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Keywords: *Burkholderia mallei*; Heat shock proteins; Host response

1. Introduction

Heat-shock proteins (hsps) are one of the most highly conserved proteins in nature and play a critical role as molecular chaperones for newly synthesized proteins in the cell (Zugel and Kaufmann, 1999). In bacteria that are stressed, such as by changes in environmental conditions, the synthesis of these proteins in the cell is up-regulated. They participate in proper folding and assembly of selected polypeptides as they come off the ribosome, play a role in transporting proteins to different locations in the cell, and play a role in the degradation of aggregated or misfolded proteins in the cell. In addition, bacterial hsps readily elicit an immune response in the host for several reasons (van Eden et al., 2005): 1) the high conservation between hsps of bacterial cells ensures that the host is primed to respond to the presence of common epitopes on the hsps; 2) under the stress of establishing an infection, the bacteria up-regulates the synthesis of hsps to make them more readily available to interact with the host's immune system; and 3) hsps associated with newly synthesized or misfolded microbial proteins can be taken up by professional antigen-presenting cells, which process both proteins through the major histocompatibility complex class I and II pathways (Shinnick, 1991; Stewart and Young, 2004; van Eden et al., 2005).

Very little is known about the hsps from *Burkholderia mallei*, the causative agent of glanders. *B. mallei* is a Gram-negative bacillus, which causes glanders primarily in horses, mules, and donkeys. Oral infections or contact with an infected animal are the most common methods of acquiring glanders because the organism can be transmitted through droplets or saliva. In horses, glanders is often manifested as a slow progressive chronic disease, but in donkeys and mules, the disease often takes an acute course with death occurring within a week to 10 days (Acha and Szyfres, 1987; Domma, 1953). Involvement of

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the lungs and upper respiratory tract is common and is manifested by a regional or diffuse pneumonia or pleuritis, and a nasal exudate, which is infectious. Cutaneous infections are manifested by enlarged and indurated (farcy) regional lymph nodes, which may rupture and suppurate. There is no effective treatment of glanders in the natural host, so animals diagnosed with glanders are isolated and destroyed. In humans, infection by *B. mallei* may be through the oral, nasal, ocular, or cutaneous routes. It was not known that *B. mallei* was highly infectious for humans until the organism was studied under laboratory conditions. It was noted that within the first year of study, half the workers became infected with glanders most likely through an aerosol route (Waag and DeShazer, 2004; Howe and Miller, 1947). In a more recent report, glanders was obtained possibly through a cutaneous route by an investigator, and once the organism was identified, a 6-month treatment with a combination of azithromycin and doxycycline was effective in resolving the infection (Srinivasan et al., 2001). Progression of the disease is similar to that seen in the natural hosts and may present as an acute localized or pulmonary form, which can lead to a fatal septicemic illness. A chronic lymphangitis and regional adenopathy may also be seen. Usually, in humans a combination of the symptoms is seen.

Our hypothesis is that the *B. mallei* GroEL (the *Escherichia coli* homologue for the large 60-kDa hsp) and DnaK (the *E. coli* homology for 70-kDa hsp) proteins, which are molecular chaperones that are from the *groESL* and *dnaK* operons, respectively, are immunogenic in a glanders patient, and because of their ability to induce a T-cell response in the host, they can potentially be used as part of a vaccine to prevent or ameliorate glanders infection in humans. In our studies reported here, we examined the host immune response of an infected patient and mice to the *B. mallei* hsps GroEL and DnaK proteins. The infected patient was the first reported human glanders case in 50 years in the United States (Srinivasan et al., 2001). The GroEL protein was found to be more immunoreactive than the DnaK protein in antisera from a patient and mice previously infected with *B. mallei*. More GroEL protein appeared to be released from the cells during growth than the DnaK protein, which may explain the host's differential immune response to these hsps from *B. mallei*.

2. Materials and methods

2.1. Antiserum, bacterial cells, and proteins

Antisera from the *B. mallei*-infected patient (Srinivasan et al., 2001) and healthy volunteer patients were obtained from the US Army Medical Research Institute of Infectious Diseases (USAMRIID, Fort Detrick, MD). Preexisting anonymous specimens were approved for purposes of this study by the USAMRIID Human Use Committee, which determined the specimens met conditions for exemption category 4 [45 CFR 46.101(b)(4)]. Mouse antisera were obtained from uninfected BALB/c mice or previously infected BALB/c mice, which had survived a low aerosol challenge of *B. mallei* ATCC 23344. *B. mallei* cells used for antibody studies were prepared as previously described (Amemiya et al., 2002). The *B. mallei* strain (pBH1-GFP) containing green fluorescent protein (GFP) was constructed as previously described (Stevens et al., 2005). Recombinant GFP was obtained from BioVision Research Products, Mountain View, CA, and chicken anti-GFP antibody was obtained from Upstate USA, Charlottesville, VA. The plague candidate vaccine F1-V was obtained from Dr. Brad Powell here at USAMRIID, and anthrax vaccine recombinant protective antigen (rPA) was obtained from Dr. Bruce Ivins (USAMRIID).

Research was conducted in compliance with the Animal Welfare Act and other federal statues and regulations relating to animals and experiments involving animals, and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.2. Cloning, overexpression, and purification of hsps

We began our cloning and sequencing of the *B. mallei groEL* and *dnaK* genes before they were completed and annotated by The Institute of Genomic Research (TIGR) at www.tigr.org/. The *B. mallei groEL* and *dnaK* genes were cloned by using polymerase chain reaction (PCR) primers with sequences obtained from the sequences of the genes from *Burkholderia pseudomallei* deposited in GenBank (AF287633 and AF016711, respectively) and *B. mallei* ATCC 23344 DNA. The *B. pseudomallei* DNA sequences (Holden et al., 2004) were used because of the close homology (99%) between the genomic sequences of *B. mallei* and *B. pseudomallei*. The sequences of the primers used for the *groEL* gene were as follows: sense, 5′-ATGGCAGCTAAAGACGTCG-3′; antisense, 5′-TTACATGTCCATGCCCAT-3′. The sequences of the *dnaK* gene primers were as follows: sense, 5′-ATGGGAAAGATCATCGGTATTGACC-3′; antisense, 5′-TCAGTCCTTCTTCACTTCCTGAAG-3′. After the PCR reaction, the respective products were purified from the agarose gel and cloned into the pCR T7/NT-TOPO vector (Invitrogen, Carlsbad, CA). The genes were sequenced and subsequently subcloned into a Multisite Gateway vector (Invitrogen), DNA sequence confirmed, and proteins overexpressed and purified by the Protein Expression and Purification Laboratory at the National Cancer Institute, Frederick, MD. The his6-MBP-tev-tag was cleaved from each protein before the final purification of the protein. The endotoxin levels (Cambrex Bio Science, Walkersville, MD) for the GroEL and DnaK preparations were 0.22 and 8 EU/mg, respectively.