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ORIGINAL ARTICLE

Immunoreaction of a recombinant nanobody from camelid single domain antibody fragment with *Acinetobacter baumannii*

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Background: *Acinetobacter baumannii*, an important nosocominal pathogen, causes various human infections like meningitis, bacteremia and pneumonia. The aim of this study was to produce nanobodies derived from camel heavy chain antibodies (HcAb) against a conserved region of the biofilm associated protein (Bap) of *A. baumannii*, by phage display technique.

Methods: A camel was immunized with the purified recombinant protein expressed from the conserved region of Bap and polyclonal antibody production was confirmed by ELISA. After RNA extraction from peripheral lymphocytes, cDNA was prepared and a phagemid library was constructed.

Results: Phage particles encoding nanobodies were produced by infecting transformed cells with a M13K07 helper phage. A total of six panning rounds were performed to select high affinity clones. Screening of high affinity monoclonal nanobodies was performed using phage-ELISA. A clone with the highest absorption in monoclonal phage-ELISA was selected for soluble expression of the desired nanobody.

Conclusions: This is the first report on the expression and production of nanobodies against Bap. Increasing trends of drug resistance have shifted the focus to the role of antibodies in diagnosis and treatment of human diseases. Similarities of the produced VHH to human VH, makes the role of this nanobody promising in immunotherapy.

Keywords: *Acinetobacter baumannii*, Biofilm associated protein, Nanobody, Phage display, VHH

Introduction

Acinetobacter spp. are gram-negative, non-motile, aerobic, non-fermenting bacteria.[1] *Acinetobacter baumannii*, an important nosocomial pathogen, causes various human infections, such as meningitis, bacteremia, pneumonia and urinary tract infections.[2–4] Its remarkable resistance to a wide range of antibiotics has made the treatment of infections very difficult. The mortality rate of infected patients is 43% and in some countries this rate has risen to 75%.[5,6] Worldwide reports of multidrug-resistant (MDR) strains of *A. baumannii* among hospitalized patients are increasing.[7] It is hypothesized that the ability of *A. baumannii* to resist antimicrobial stressors, antibiotics or cleaning is due to its ability to form biofilms.[8] Studies suggest that a positive relationship exists between *A. baumannii's* MDR and biofilm formation.[9] Biofilm-associated protein (Bap), a surface protein with a very high molecular mass and central tandem repetitions, is associated with bacterium virulence and drug resistance.[10] This protein with 8620 amino acids is one of the largest proteins identified. It is one of the most acidic proteins with an isoelectric point of about 3.[11] Bap is the surface structure involved in adherence of *A. baumannii* to both normal human bronchial epithelial cells and human neonatal keratinocytes.[12] Bioinformatics tools have identified a core domain of seven repeat modules; namely, A to G, of which D, B, C and A are predominant. Because of their three-dimensional structure and antigenic properties, they are nominated as appropriate candidates for vaccination and antibody production. In a previous study we demonstrated that four regions of Bap from *A. baumannii* were effective antigens. All regions were predicted to be conserved and functional in the native protein.[13] Previous studies have reported high antibody titers in mice after immunization with the conserved region of Bap construct-II consisting of 371 amino acids.[14] Some of the main concerns in *A. baumannii* infection include the fast growing populations of MDR strains of the bacterium, its remarkable survival ability in nosocominal environments, and the time consuming and expensive procedures used for its identification.[15] A previous study[14] proved the immunogenicity of *A. baumannii* Bap, therefore, in this study, we attempted to produce a recombinant nanobody derived from camel heavy chain antibodies

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