Effects of Immunoglobulins against Specific Exosporium Proteins on Spore Germination of *Bacillus anthracis in vitro*

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**Background**

*Bacillus anthracis* is a rod-shaped, gram (+) spore-producing bacteria that is the causative agent of Anthrax – a deadly disease and potential bioterrorism threat. The infectious particles are spores which must germinate in vivo and produce toxins in order to cause illness. The spores are composed of a single bacterial cell surrounded by a multilayer protein shell – the outermost of which is the exosporium. The exosporium consists of the inner basal layer and an outer hair-like nap layer, that is comprised primarily by the immunodominant glycoprotein BclA.

The host’s immune system produces antibodies against exosporium proteins upon vaccination with whole-spore preparations. Currently, the primary vaccine used in the United States results in antibody protection against a critical *B. anthracis* protein called Protective Antigen (PA). Brahmbhatt et al.¹ found that boosting with anti-BclA antibodies after a primary immunization against PA offered full protection from disease. Similarly, vaccination with PA with the BclA protein is a rod-shaped, gram (+) spore-producing bacteria that is the causative agent of Anthrax – a deadly disease and potential bioterrorism threat. The infectious particles are spores which must germinate in vivo and produce toxins in order to cause illness. The spores are composed of a single bacterial cell surrounded by a multilayer protein shell – the outermost of which is the exosporium. The exosporium consists of the inner basal layer and an outer hair-like nap layer, that is comprised primarily by the immunodominant glycoprotein BclA.

Studies have shown that anti-spore antibodies can delay or inhibit spore germination in vitro. However, how anti-sera raised against specific exosporium proteins will effect germination remains unclear.

The objective of this study is to assess the effects of polyclonal antibodies raised against specific exosporium proteins on spore germination of *Bacillus anthracis in vitro*. We hypothesize that, like anti-spore antibodies, anti-sera raised against specific exosporium proteins will delay germination.

**Materials and Methods**

**Heat Inactivation Assay**

- **Western Blot:** Antibodies against exosporium proteins (α-BclA, α-BclB, α-BxpB and α-BAS3503) bound to their respective proteins, when present.

**Results**

**Western Blots** Probed with antibodies against specific exosporium proteins

- *B. anthracis* Spore EM courtesy of Dr. Brian Thompson.

**Heat Inactivation Assay**

- Spores incubating in BHI for 0 min. were not inactivated by heat. In contrast, spores incubating in BHI for 30 min. showed no colony growth when inactivated by heat.

**Summary and Conclusions**

- Previous studies showed that antibodies towards whole-spore preparations delayed spore germination *in vitro* for *Bacillus anthracis*. Therefore, immunization with particular exosporium proteins may be useful in improving vaccination success.

- In our experiments we observed that:
  - Western Blot: Antibodies against exosporium proteins (α-BclA, α-BclB, α-BxpB and α-BAS3503) bound to their respective proteins, when present.
  - Heat Inactivation: There were no clear differences in % germination through time between control and treatment groups.

- Antibodies to specific exosporium proteins were not found to effect germination of *Bacillus anthracis* spores *in vitro*.