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Development of a mAb library targeting the capsular polysaccharide of *Burkholderia pseudomallei*

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ABSTRACT

Burkholderia pseudomallei, the causative agent of melioidosis, produces a high molecular weight capsular polysaccharide (CPS) comprised of an unbranched homopolymer of 1,3-linked 2-*O*-acetyl-6-deoxy-β-D-*manno*heptopyranose residues. Our laboratory has previously produced a CPS specific IgG3 monoclonal antibody (mAb), 3C5, and determined that it is able to provide passive protection in a murine model of pulmonary melioidosis. In addition, the mAb was used to detect shed CPS in melioidosis patient samples and was successfully incorporated into a prototype Active Melioidosis Detect™ Lateral Flow Immunoassay (AMD LFI) through a collaboration with our laboratory and InBios International, Inc. While a successful prototype was produced using mAb 3C5, background issues attributed to IgG3 self association lead us to pursue making additional antibodies of alternate isotypes. We have recently isolated several murine IgG1 mAbs targeting CPS by immunizing BALB/c mice with a CPS glycoconjugate. The new library of mAbs show a range in CPS binding affinities, with one mAb in particular, 4C4, having a considerably higher affinity for CPS compared to our previous IgG3 mAb. An updated LFI incorporating a new CPS-specific IgG1 displays high CPS sensitivity with greatly reduced background signal, highlighting its potential as a sensitive and specific rapid diagnostic for melioidosis.

INTRODUCTION

Burkholderia pseudomallei is a gram negative bacillus endemic to southeast Asia and northern Australia and is the causative agent of melioidosis. Culture of *B. pseudomallei* from patient samples remains the "gold standard" for the diagnosis of melioidosis. Early antibiotic treatment of melioidosis is essential for patient survival. Unfortunately, culture is slow, requiring sample collection of blood, urine, pus, and/or other body fluids that then must be cultured for at least 48 hours due to the low levels of *B. pseudomallei* found in these fluids. Alternative diagnostics have been developed, including indirect hemagglutination assays (IHA), PCR, latex agglutination, immunofluorescence assays, and ELISA, however, these assays suffer from low sensitivity, require expensive equipment, or other drawbacks. For example, much of the population in endemic areas is seropositive for *B. pseudomallei* antibodies, which makes IHA a poor indicator of infection.

Lateral flow antigen capture assays present an opportunity for a more sensitive, rapid, and inexpensive diagnostic by detecting antigen that is actively shed in patient samples to identify active disease without relying on cultured samples. *In vivo* Microbial Antigen Discovery (InMAD) has identified the capsular polysaccharide (CPS) of *B. pseudomallei* as an encouraging diagnostic target. Our laboratory initially produced a monoclonal antibody, mAb 3C5, and, with InBios International, incorporated it into an Active Melioidosis Detect™ Lateral Flow Immunoassay (AMD LFI). The assay showed high sensitivity for CPS, but was limited by background issues resulting from IgG3 self association. In an attempt to decrease background while maintaining or increasing sensitivity, our laboratory has produced and characterized a library of 12 additional mAbs that target CPS to potentially replace or be used in conjunction with mAb 3C5. Of these, five of the top candidates were further screened for potential use in a lateral flow diagnostic assay.

METHODS

Immunization of mice and production of CPS mAbs. BALB/c mice were immunized with CPS conjugated to BSA that was kindly provided by Drs. Paul J. Brett and Mary N. Burtnick. Hybridoma cell lines specific for CPS were identified and grown in an Integra CL 1000. MAbs were purified by affinity chromatography over recombinant Protein A Sepharose beads.

Western blot analysis. Bacterial cells or purified CPS were transferred to a nitrocellulose membrane and probed with CPS mAbs. Bound CPS mAbs were then detected using horseradish peroxidase (HRP) labeled goat-anti-mouse IgG followed by a chemiluminescent substrate.

METHODS CONTINUED

ELISA analysis. Purified CPS was serial diluted in microtiter plates that were coated overnight with CPS mAbs. Bound CPS was then detected using horseradish peroxidase (HRP) labeled CPS mAbs followed by a tetramethylbenzidine substrate. Results were corrected for background and analyzed by linear regression for an OD (450 nm) value of 0.3.

Affinity analysis. Using a Biacore X100, surface plasmon resonance was used to determine the affinity of each mAb in our library. Biotintylated CPS was immobilized to a streptavidin sensor chip and probed with CPS mAbs.

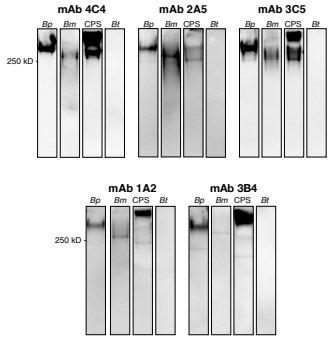


Figure 1. Characterization of CPS-specific mAb library. 5 anti-CPS mAbs are reactive with *B. pseudomallei* (*Bp*) strain 1026b, *B. mallei* (*Bm*) strain AGD0000503, and purified CPS. mAbs are not reactive to avirulent *B. thailendensis* (*Bt*) (lacks CPS).

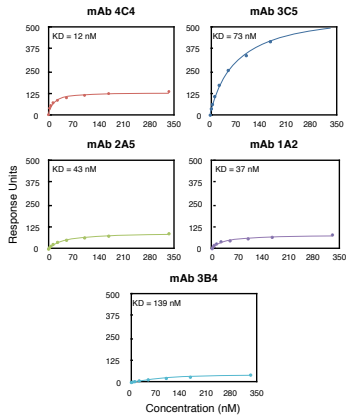
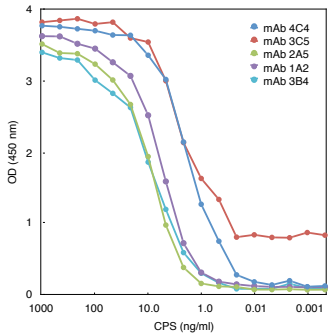


Figure 2. Affinity data of CPS-specific mAb Library. Biacore sensorgrams showing five anti-CPS mAbs binding to purified CPS immobilized on the sensor chip surface. A smaller dissociation constant (KD) corresponds to a higher affinity.



mAb	Subclass	Limit of Detection (ng/mL)
4C4	IgG1	0.25
3C5	IgG3	0.36
2A5	IgG1	1.56
1A2	IgG1	0.72
3B4	IgG1	0.7

Figure 3. Sensitivity data of CPS-specific mAb Library. Normalized CPS antigen capture ELISA data showing five anti-CPS mAbs binding to purified CPS. The limit of detection is measured at an OD (450 nm) value of 0.3.

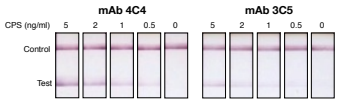


Figure 4. Lateral flow immunoassay images. LFI was tested with purified CPS (ng/ml) diluted in normal human sera. An updated LFI incorporating mAb 4C4 is more sensitive than the original assay with mAb 3C5.

CONCLUSIONS

- To improve upon the current AMD LFI, a library of non-IgG3 mAbs targeting *B. pseudomallei* CPS were generated.
- CPS mAbs are reactive with *B. pseudomallei* and *B. mallei*, but not to avirulent *B. thailandensis* (lacks CPS).
- The IgG1 mAb, 4C4, has a higher affinity than the original IgG3 mAb (3C5).
- A new lateral flow immunoassay constructed with the IgG1 mAb 4C4 has reduced background and has greater sensitivity (at least 0.5 ng/ml) compared to the IgG3 prototype.

ACKNOWLEDGEMENTS

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