Human Single-Chain Fv Antibodies Against *Burkholderia mallei* and *Burkholderia pseudomallei*

NIANXIANG ZOU, TAMARA NEWSOME, BINGJIE LI, SHIEN TSAI, AND SHYH-CHING LO[1]

Department of Environmental and Infectious Disease Sciences, American Registry of Pathology, Armed Forces Institute of Pathology, Washington, DC 20306

Much effort has been devoted to the development of mouse monoclonal antibodies that react specifically with *Burkholderia mallei* and *Burkholderia pseudomallei* for diagnostic and/or therapeutic purposes. Our present study focused on the screening of a phage-displayed nonimmune human single-chain Fv (scFv) antibody library against heat-killed *B. mallei* and *B. pseudomallei* for the generation of human scFv antibodies specific to the two pathogenic species of bacteria. Using two different panning procedures, we obtained seven different scFv phage antibodies that interacted with the heat-killed whole bacterial cells of *B. mallei* and *B. pseudomallei*. Our results demonstrate that panning of a human scFv antibody library against heat-killed whole bacterial cells may provide a valuable strategy for developing human monoclonal antibodies against the highly pathogenic bacteria. Exp Biol Med 232:550–556, 2007

Key words: single-chain Fv; scFv; *Burkholderia mallei*; *Burkholderia pseudomallei*

Introduction

Burkholderia mallei and *Burkholderia pseudomallei* are gram-negative facultatively anaerobic pathogenic bacteria. *Burkholderia mallei*, the causative agent of glanders, is a host-adapted Burkholderia species. It is closely related to *B. pseudomallei*, a human pathogen that exists predominantly in subtropical areas and is the causative agent of melioidosis (1). Both species can cause infections in humans through direct inoculation into wounds and skin abrasions or inhalation of contaminated material. Despite aggressive regimens with certain antibiotics to treat the infections, the mortality rate of melioidosis remains very high (2, 3). Due to the possibility of aerosol dissemination and the highly pathogenic nature of these 2 species in humans, *B. mallei* and *B. pseudomallei* may potentially be misused as biologic warfare agents (4). Thus, efficient identification and therapeutic approaches are needed against infections of *B. mallei* and *B. pseudomallei*.

Mouse monoclonal antibodies specific to the capsular polysaccharide, lipopolysaccharide, or proteins of *B. pseudomallei* have been developed; studies on passive protection against *B. pseudomallei* infection in the mouse model have also been conducted (5). Mouse monoclonal antibodies specific to the *B. pseudomallei* lipopolysaccharide have been used for the immunologic detection and identification of *B. pseudomallei* (6–8). Monoclonal antibodies against lipopolysaccharides apparently have limitations in distinguishing between closely related Burkholderia species, especially among *B. mallei*, *B. pseudomallei*, and *Burkholderia thailandensis*, a closely related nonpathogenic species. Therefore, monoclonal antibodies with higher performance in affinity and specificity to the pathogenic species of Burkholderia bacteria are highly desirable for bacterial detection assays and passive immune therapies.

Phage-displayed single-chain Fv (scFv) library technology is a powerful approach to quickly develop monoclonal antibodies in the scFv format to a wide variety of antigens (9–11). In this approach, coding sequences of antibody light chain and heavy chain variable regions from pools of peripheral blood B lymphocytes are amplified and linked together by linker sequences, and the resulting scFv genes are cloned into an M13 phage–based expression vector (12). The scFv antibodies fused with an M13 phage protein are expressed on the phage surface for convenient screening (panning) procedures. Using phage-displayed human scFv libraries, one can directly obtain the coding sequences of variable regions of human antibodies. This greatly facilitates antibody manipulation procedures.

The development of mouse scFv antibodies against *B.*

This work is supported in part by a Defense Threat Reduction Agency grant (2.10014_05_AF_B).

[1] To whom correspondence should be addressed at AFIP, Bldg. 54, Room 3025, 6825 16th Street, NW, Washington, DC 20306. E-mail: los@afip.osd.mil

Received August 23, 2006.
Accepted November 6, 2006.

1535-3702/07/2324-0550$15.00

pseudomallei exotoxin has recently been published (13); however, development of human scFv antibodies to *B. pseudomallei* or *B. mallei* has not been reported. In the present study we report screening of an existing nonimmune human scFv library (14) using 2 different approaches against heat-killed *B. mallei* and *B. pseudomallei* whole bacteria and characterization of the resulting scFv antibodies.

Materials and Methods

Bacterial Culture Conditions and Sample Preparation. *Burkholderia mallei* (ATCC 23344), *B. pseudomallei* (ATCC 23343), *B. thailandensis* (ATCC 700388), *Burkholderia cepacia* (ATCC 700070), *Burkholderia stabilis* (ATCC BAA-244), and *Burkholderia vietnamiensis* (ATCC BAA-248) were grown separately in Luria-Bertani (LB) medium at 37°C overnight with shaking. Bacterial cells were collected by centrifugation at 4000 *g* for 10 mins and resuspended in 1× phosphate-buffered saline (PBS). The bacteria in PBS were killed by heating at 65°C for 45 mins. Each heat-killed whole bacterial sample was quantified by total protein concentration with detergent-compatible protein assay reagents (Bio-Rad Laboratories, Hercules, CA).

Rescue of scFv Phage. The human nonimmune scFv library in the form of frozen *Escherichia coli* cells was kindly provided by Dr. Kim D. Janda of the Scripps Institute, La Jolla, California. The scFv phage library used for panning experiments was prepared as described (14). Briefly, 10 ml of the expanded *E. coli* scFv library glycerol stock ($\sim 5 \times 10^{10}$ cells) was inoculated into 1 liter of SB medium (30 g tryptone, 20 g yeast extract, 10 g MOPS, 1 liter ddH_2O; pH 7.0) containing 2% glucose, 50 μg/ml carbenicillin, and 20 μg/ml tetracycline and incubated by shaking at 37°C until $OD_{600} = 0.5–0.7$. Then 4×10^{13} pfu of VCSM13 helper phage and 2 ml of 0.5 *M* isopropyl β-D-thiogalactopyranoside (IPTG) were added to the culture, which was incubated without shaking at room temperature for 30 mins. The culture was then diluted to 5 liters by SB medium containing 50 μg/ml carbenicillin, 20 μg/ml tetracycline, and 0.5 m*M* IPTG and incubated at 30°C for 2 hrs. Kanamycin was added to 70 μg/ml, and the culture was incubated by shaking at 30°C overnight. The library phage was precipitated from culture supernatant by adding 20% volume of 40% PEG-8000 containing 2.5 *M* NaCl. The pellet was resuspended in 5 ml of PBS + 2% bovine serum albumin (BSA) + 0.7% dimethyl sulfoxide, aliquoted into 0.5-ml samples, and stored at −70°C.

Panning of scFv Phage Library. The procedure for panning of the human phage library against the free (nonimmobilized) heat-killed whole bacterial cells was performed as follows. In each panning whole bacterial suspension in the designated amounts was mixed with 1×10^{13} pfu of the library phage (first panning) or amplified phage preparation from the previous panning in 1 ml of PBS + 2% BSA + 2% skim milk at room temperature for 2 hrs. Bound phage particles were pelleted with bacterial cells at 5000 rpm for 5 mins at room temperature. The pellet was resuspended in 1 ml of PBS + 0.1% Tween-20 and pelleted again at 5000 rpm for 5 mins at room temperature. After repeating this procedure 10 times, the pellet was resuspended in 0.5 ml of elution buffer (100 m*M* HCl, adjusted to pH 2.2 with solid glycine and containing 0.1% BSA) to elute the bound phage from the bacterial cells. The elute was neutralized with 30 μl of 2*M* Tris base (pH not adjusted) and used to infect log-phase *E. coli* XL1-Blue cells for scFv phage rescue and amplification. A small portion of the infected *E. coli* cells was plated on LB plates containing carbenicillin (50 μg/ml) and tetracycline (20 μg/ml) to calculate the phage recovery of each panning. The amplified scFv phages were then used to start the next round of panning.

The procedure for panning of the human phage library against the heat-killed bacteria immobilized on the immuno sticks was as follows. Nunc Immuno Sticks (Nalge Nunc International, Rochester, NY) were coated with designated amounts of heat-killed *B. mallei* cells in 0.1 *M* Na_2CO_3 (pH 9.6) at room temperature overnight. After coating, the sticks were blocked with PBS containing 2% BSA and 2% skim milk at room temperature for 4 hr and then stored at −20°C before use. Library phage (1×10^{13} pfu) or amplified phage preparation from the previous panning in PBS with 2% BSA and 2% skim milk was incubated with the immuno sticks at 4°C overnight with gentle rocking. Unbound scFv phage was removed from the immuno sticks by 10 washes with PBS + 0.1% Tween-20 (PBST) followed by 10 washes with PBS. The specifically bound phage was eluted by incubation with 0.5 ml of elution buffer at room temperature for 10 mins. The elute was neutralized with 30 μl of 2*M* Tris base (pH not adjusted) and was used to infect log-phase *E. coli* XL1-Blue cells for scFv phage rescue and amplification. A small portion of the infected *E. coli* cells was plated on LB plates containing carbenicillin (50 μg/ml) and tetracycline (20 μg/ml) to calculate the phage recovery of each panning. The amplified scFv phages were used to start the next round of panning.

Screening for scFv Positive Phage Antibodies by ELISA. To screen for the scFv phage antibodies specific to *B. mallei* and/or *B. pseudomallei*, enzyme-linked immunosorbent assays (ELISAs) were performed using the detection module for the recombinant phage antibody system (Amersham Pharmacia Biotech, Piscataway, NJ). Briefly, *E. coli* XL1-Blue colonies harboring recombinant phagemids were cultured individually in 1.5-ml Eppendorf tubes and rescued to produce recombinant scFv phage. The scFv phage lysates were incubated with PBS + 2% skim milk at a 1:1 ratio at room temperature for 30 mins. One hundred microliters of the above mixture was added to each well of ELISA plates coated with heat-killed whole-cell samples. The positive control for the ELISA reaction was used with the positive control antigen and positive control

Table 1. ELISA Results of Panning Against Immobilized *B. mallei* Cells

	Clones tested	Positive clones[a]	Positive rates
Panning round 1	47	0	0
Panning round 2	144	71	49.3%
Panning round 3	143	106	74.1%
Panning round 4	48	47	97.9%

[a]ScFv phage clones with ELISA OD_{405} readings 5X or greater than background ($OD_{405} = 0.05$) were considered positive.

phage provided by the company. After incubation for 2 hrs, ELISA plates were washed with PBST 9 times with an EL403H Microplate Autowasher (Bio-Tek Instruments, Winooski, VT). Secondary antibody interactions were accomplished with 100 μl of a horseradish peroxidase (HRP)–conjugated anti-M13 monoclonal antibody diluted at 1:5000 in PBS + 2% skim milk. After incubation for 1 hr at room temperature and washing with PBST, 100 μl of 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid peroxidase substrate solution was added to each well. After incubation at room temperature for 1 hr, the absorbance was read at 405 nm on a V max kinetic microplate reader (Molecular Devices, Sunnyvale, CA).

Phage Antibody Characterization by DNA Sequencing. ScFv phage antibodies that showed an OD_{405} reading of 5-fold or higher compared with the negative control were chosen for a repeated ELISA to eliminate false positives. The scFv gene of individual ELISA-positive phage was amplified by PCR with the flanking primers pelB5 (5′-ATG AAA TAC CTA TTG CCT ACG-3′) and flag3 (5′-GTC ATC GTC ATC TTT GTA GTC-3′). The PCR products were gel-purified and sequenced from both ends with primers pelB5 and flag3.

Immunoblot. Heat-killed whole-bacteria samples were lysed with 1% SDS. One aliquot of each lysis was treated with proteinase K to remove proteins. Lysates were separated by SDS-polyacrylamide gel (14 cm × 12 cm × 0.75 cm) electrophoresis (PAGE) and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked with 2% skim milk in PBS and cut into 4-mm strips. Each strip was incubated with individual scFv phage antibody diluted in PBS + 2% skim milk at room temperature for 2 hrs. The strips were washed 6 times with PBS + 0.1% Tween-20 and incubated at room temperature with HRP-conjugated anti-M13 monoclonal antibody diluted 1:2000 in PBS + 2% skim milk. The strips were washed with PBS + 0.1% Tween-20, incubated with the ECL reagent (KPL, Gaithersburg, MD), and exposed to X-ray films.

Results

Panning and ELISA Screening Against Heat-Killed *B. mallei* and *B. pseudomallei*. The human scFv phage library was panned in 4 separate screening experiments against heat-killed whole bacterial cells of *B. mallei* and *B. pseudomallei*, free in suspension and immobilized on immuno sticks, respectively.

For panning with heat-killed whole bacterial cells in suspension, the nonimmobilized bacterial cells served as the solid phase. The phage library (1×10^{13} pfu) was incubated with bacterial suspension at total bacterial protein quantities of 100 μg, 50 μg, 20 μg, and 20 μg for panning rounds 1, 2, 3, and 4, respectively. No significant increase in the yield of scFv phage after each round of panning was observed using either *B. mallei* or *B. pseudomallei* cells compared with that of the previous round of panning. To screen for scFv phage clones positive for *B. mallei* or *B. pseudomallei* binding, we examined 94 scFv phagemid-containing *E. coli* colonies each from the titration plates from panning round 4 against *B. mallei* and *B. pseudomallei*. The individual scFv phages were grown, rescued by helper phage VCSM13 to produce scFv phage antibodies, and assayed against heat-killed *B. mallei* or *B. pseudomallei* cells coated on 96-well ELISA plates. Out of each set of 94 *E. coli* colonies studied, we obtained 20 and 21 clones that produced scFv phage reacting positively ($OD_{405} > 0.25$) with *B. mallei* and *B. pseudomallei,* respectively. In cross-examination by ELISAs, all of the 20 scFv phage clones obtained by panning against *B. mallei* also strongly reacted with *B. pseudomallei*, while none of the 21 svFv phage clones obtained by panning against *B. pseudomallei* cross-reacted with *B. mallei*.

In panning experiments using immuno sticks, 50 μg, 10 μg, 5 μg, and 5 μg of *B. mallei* or *B. pseudomallei* were immobilized on immuno sticks for panning rounds 1, 2, 3, and 4, respectively.

A 30-fold increase in the yield of scFv phage was observed after 4 rounds of panning against *B. mallei* whole cells immobilized on the immuno sticks as target antigens. Table 1 shows the number of *E. coli* colonies that were grown and rescued for ELISA from titration plates after each round of panning. After the first round of panning, 0 of the 47 scFv phage clones (0%) examined was found to produce phage that would react positively with *B. mallei*. In comparison, after the second round of panning, 71 of 144 *E. coli* colonies (49.3%) examined were found to produce scFv phage that reacted positively with *B. mallei*. Moreover, 106 of 143 scFv phage clones (74.1%) examined from the third round of panning and 47 of 48 scFv phage clones (97.9%) examined from the fourth round of panning were found to produce *B. mallei*-reacting phage. All of the scFv phage clones selected by panning against *B. mallei* showed low or undetectable cross-reaction with *B. pseudomallei* in ELISA.

Our panning against *B. pseudomallei* immobilized on the sticks failed to produce any positively reacting scFv phage clones that reacted positively against *B. pseudomallei* in ELISA screening.

Sequencing Analysis of the Selected scFv Phage. The scFv genes of selected positive phage antibodies were amplified by PCR and sequenced. All of the 20

Table 2. Studies of Positive scFv Phage Clones Selected by Panning Against *B. mallei* Immobilized on Immuno Sticks

		Average ELISA reading (OD_{405})		
Sample group	Member in group	Anti-BM	Anti-BP	scFv size (bp)
1F3	22	1.05	0.074	738
4F1	40	1.07	0.049	645
2C5	1	0.76	0.051	744
3G5	1	1.296	0.061	738
4G10	2	1.490	0.046	663
Positive control		1.44		

positive phage clones obtained from panning against heat-killed free *B. mallei* cells were found to contain the same scFv gene sequence—they were apparently 1 identical scFv phage antibody (designated BM1). Similarly, all 21 positive phage clones obtained from panning against heat-killed *B. pseudomallei* cells were found to contain an identical scFv gene sequence; thus, all clones represented one identical scFv phage antibody (designated BP1). We sequenced scFv genes of 72 positive phage antibodies derived from panning against heat-killed *B. mallei* cells immobilized on immuno sticks. Sequence analysis of the scFv genes of these phage revealed that there were 5 different scFv phage antibodies (designated 1F3, 2C5, 3G5, 4F1, and 4G10 in Table 2). scFv phage antibodies 1F3 and 4F1 had multiple members; 2C5 and 3G5 each had a single member; and 4G10 had 2 members. All 5 scFv phage antibodies reacted specifically with *B. mallei* and showed little cross-reaction with *B. pseudomallei* in ELISA (Table 2). The GenBank entries for the 7 scFv genes are DQ915095–DQ915101.

A sequence comparison with Ig sequences using IgBlast (http://www.ncbi.nlm.nih.gov/igblast/) revealed that 1F3 and 3G5 contained VH1 and VL κ3 subfamily, 2C5 contained VH6 and VL κ3 subfamily, BM1 contained VH3 and VL κ2 subfamily, and BP1 contained VH1 and VL λ subfamily. The VH regions of 4F1 and 4G10 are almost identical (2 amino acid difference) and produced no hits in immunoglobulin databases. The VL regions of 4F1 and 4G10 differ markedly and both belong to the κ3 subfamily.

Immunoblot Analysis. To examine the nature of antigens recognized by the scFv phage antibodies obtained above, we propagated each of the phage antibodies and adjusted their final titers to 1×10^{11} pfu/ml in PBS + 2% BSA. Immunoblot analysis was performed on membrane strips prepared by proteins transferred from SDS-PAGE of *B. mallei* and *B. pseudomallei* lysates with or without proteinase K pretreatment. The results of immunoblot experiments with scFv phage antibodies BM1, BP1, 1F3, 2C5, 3G5, 4F1, and 4G10 are shown in Figure 1.

The phage antibody BM1, which was obtained through panning with heat-killed *B. mallei* whole bacterial cells free in suspension, generated a strong multiband signal ranging from approximately 20 to 130 kDa on the membrane strips made from both *B. mallei* and *B. pseudomallei* lysates (Fig. 1). However, the strong signal was highly sensitive to pretreatment of the bacterial lysates with proteinase K, indicating that the nature of the epitope recognized by the monoclonal phage antibody is most likely composed of a specific peptide sequence. The phage antibody BP1, which was obtained through panning against free heat-killed *B. pseudomallei* whole bacterial cells, did not produce any signals on the membrane strips from either *B. pseudomallei* or *B. mallei* lysates. The phage antibodies 2C5, 4F1, and 4G10 also did not generate any positive signals on the membrane strips from either *B. pseudomallei* or *B. mallei* lysates in immunoblot, despite their strong reactivity to *B. mallei* whole bacterial cells in ELISA. Both of the phage antibodies 1F3 and 3G5 reacted positively with a single protein band of approximately 92 kDa on the membrane strips of *B. mallei* lysate in the immunoblot experiment (Fig. 1). They also weakly cross-reacted with a band on the membrane strip of the *B. pseudomallei* lysate.

Reactivity with Other Nonpathogenic Burkholderia Species. We examined the reactivity of the phage antibodies to some other species of Burkholderia by ELISA. Equal amounts of heat-treated whole bacterial samples and SDS lysates of *B. cepacia*, *B. mallei*, *B. pseudomallei*, *B. stabilis*, *B. thailandensis*, and *B. vietnamiensis* were coated on an ELISA plate. The phage antibody BM1 reacted positively with both whole-cell bacteria and SDS lysates of all of the tested Burkholderia species with comparable intensities except that of *B. thailandensis*, which showed a complete lack of reaction. The phage antibodies 1F3 and 3G5 reacted positively with both the whole-bacteria and SDS lysates of *B. mallei* and weakly cross-reacted with those of *B. pseudomallei* and other Burkholderia species tested. The phage antibody BP1 reacted positively only with whole bacterial cells of *B. pseudomallei* but not with its SDS lysate. Similarly, phage antibodies 2C5, 4F1, and 4G10 reacted positively only with the whole bacterial samples of *B. mallei* but not with its SDS lysate (Fig. 2). The ELISA results agreed with results obtained in immunoblot assays. We also performed ELISAs against 2 gram-negative bacteria, *Pseudomonas aeruginosa* and *E. coli*, whole cells and SDS lysates with each scFv antibody. None of them cross-reacted with these 2 non-Burkholderian species of bacteria (data not shown).

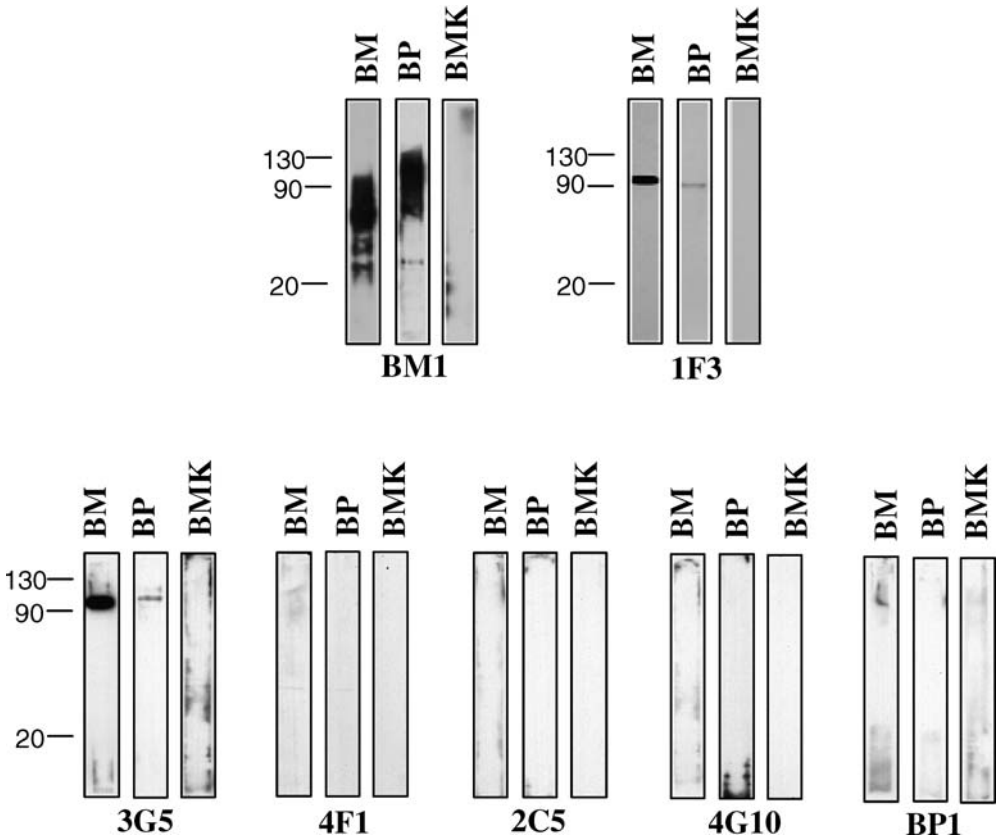

Figure 1. Analysis of reactivity of scFv antibodies with immunostrips. The immunostrips (5 × 0.4 cm) were made by separation of lysates of *B. mallei* (BM) or *B. pseudomallei* (BP) on SDS PAGE followed by transfer to nitrocellulose membrane. The scFv antibody used in each assay is indicated below the strips. The molecular weight of each positive signal or signal range was determined in separate immunoblot experiments including a molecular marker. BMK, proteinase K–treated lysate of *B. mallei*; BPK, proteinase K–treated lysate of *B. pseudomallei*.

Discussion

Developed in the early 1990s phage-display scFv libraries have become an important resource for developing antibodies for a wide variety of antigens of both pure and complex nature. Studies using scFv libraries to develop antibodies using spores and whole bacterial cells have been reported (15–18). Here we report a study using heat-killed whole bacteria of *B. mallei* and *B. pseudomallei* as screening targets to develop antibodies from an existing nonimmune scFv phage library (14).

Two different screening approaches were performed: (i) panning against heat-killed whole bacteria in suspension and (ii) panning against heat-killed whole bacteria immobilized on immuno sticks. In the first approach, heat-killed *B. mallei* and *B. pseudomallei* whole bacteria were used as both the target antigens and the solid phase. Panning was performed by incubating the scFv phage library with heat-killed whole bacterial cells. Unbound phage were removed by washing and centrifugation. In the second approach, the scFv phage library was panned against heat-killed *B. mallei* and *B. pseudomallei* whole bacteria immobilized on immuno sticks.

Four rounds of pannings were performed against whole-cell suspensions of *B. mallei* or *B. pseudomallei*. For panning against each species, we sequenced 20 and 21 ELISA positive phage clones obtained from panning against *B. mallei* and *B. pseudomallei*, respectively. Sequence analysis revealed that all of the ELISA-positive phage clones selected against each Burkholderia species produced identical scFv phage antibodies. Hence, 1 scFv antibody each to *B. mallei* (BM1) and *B. pseudomallei* (BP1) was produced with this approach against *B. mallei* and *B. pseudomallei*, respectively.

Phage antibody BM1 reacted strongly with molecules of a wide range of molecular weight and produced a smear-like immunoblot pattern (Fig. 1). The BM1-reacting bands were abolished by pretreating the bacterial lysates with proteinase K, indicating that the nature of the binding epitope is protein or protein-associated moieties. The BM1 also reacted strongly in ELISAs with some other Burkholderia species including *B. pseudomallei*, *B. cepacia*, *B. stabilis*, and *B. vietnamiensis* (Fig. 2). It did not cross-react with *P. aeruginosa*, which belongs to a different genus phylogenetically close to Burkholderia. However, surprisingly BM1 also did not interact with *B. thailandensis* (Fig. 2), although *B. thailandensis* is genetically closer to *B. mallei* and *B. pseudomallei* than any of the other Burkholderia species examined. Interestingly, by immunizing mice with *B. mallei* and *B. pseudomallei* and screening mouse hybridomas in a separate study, we also obtained a

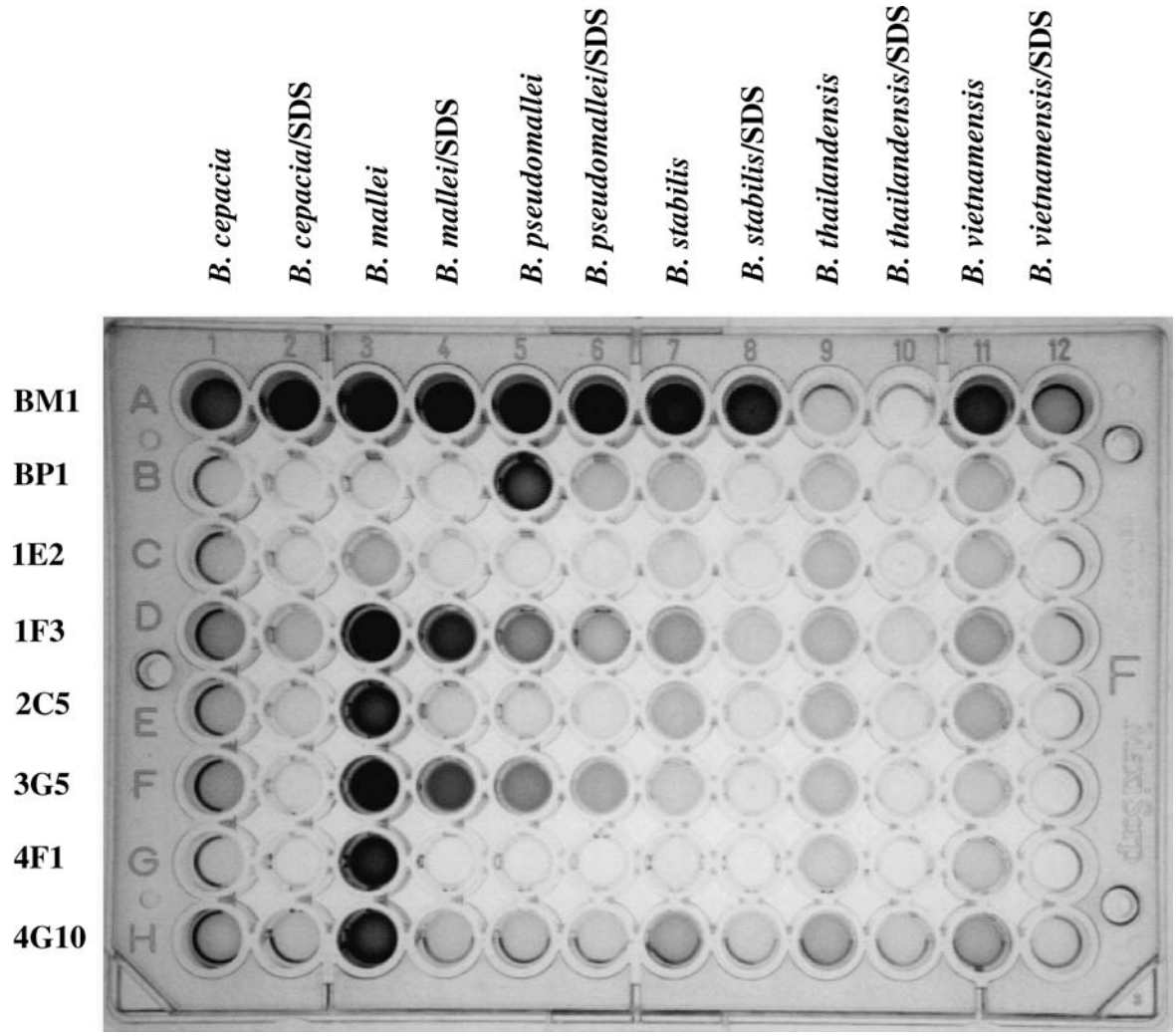

Figure 2. ELISA of phage antibodies against whole bacteria and SDS lysate of various Burkholderia species. Equal amounts of whole bacterial sample of Burkholderia species without and with SDS treatment were separately coated on each well of the specified columns. A specified scFv phage antibody (1:50 dilution) was added in each row of wells. The OD_{405} was obtained with a mean background reading of 0.05. A reading of 0.2 or higher was considered positive in this study. BM1 reacted positively with both the whole bacteria and the SDS lysates of all Burkholderia species tested ($OD_{405} = 0.2–1.0$), except *B. thailandensis*. BP1 reacted positively with whole bacteria of *B. pseudomallei* ($OD_{405} = 0.4$) but not with the SDS lysate ($OD_{405} = 0.06$). 1F3 and 3G5 reacted positively with both whole bacteria and SDS lysate of *B. mallei* ($OD_{405} = 0.4–1.0$) and cross-reacted weakly with *B. pseudomallei* and other Burkholderia species tested ($OD_{405} = 0.1–0.2$). 2C5, 4F1, and 4G10 also reacted positively with whole bacteria of *B. mallei* ($OD_{405} = 0.2–0.3$) but not with the SDS lysate ($OD_{405} \leq 0.1$). 1E2, an scFv obtained from panning with immuno sticks, weakly reacted with *B. mallei* ($OD_{405} = 0.1$).

panel of mouse monoclonal antibodies that produced a similar unique immunoblot pattern. These mouse monoclonal antibodies also reacted strongly with all tested Burkholderia species except *B. thailandensis*. Furthermore, the epitopes recognized by these mouse monoclonal antibodies were sensitive to proteinase K treatment. We believe that this group of monoclonal antibodies, as well as BM1, reacts with an epitope that is present on many Burkholderia proteins with a wide range of molecular weights. This prominent Burkholderia epitope had evidently "selected," in high efficiency, the specific phage clone of BM1 that was expanded quickly in the panning process.

The phage antibody BP1 was strongly positive to *B. pseudomallei* in ELISAs while negative to other Burkholderia species tested. However, BP1 showed negative interaction in SDS-PAGE/immunoblot analysis and in ELISA with SDS lysates as target antigens (Fig. 2), suggesting that it may recognize a conformational epitope.

Four rounds of panning were performed against heat-killed whole bacteria of *B. mallei* or *B. pseudomallei* immobilized on immuno sticks. In panning against *B. mallei*, 5 different positive phage clones were obtained (1F3, 2C5, 3G5, 4F1, and 4G10). The phage antibodies 1F3 and 3G5 each recognized a single protein band from *B. mallei* and weakly cross-reacted with a protein band from *B. pseudomallei*. Phage antibodies 2C5, 4F1, and 4G10 did not show any reaction in the SDS-PAGE/immunoblot, although they showed reactivity in the ELISA with whole bacterial cells (Fig. 2), suggesting that they too might be recognizing conformational epitopes. In panning against *B. pseudomal-*

lei immobilized on immuno sticks, we failed to produce any positive phage antibodies. The reason for this is not clear, but we speculate that whole-cell bacteria of the particular *B. pseudomallei* strain (ATCC 23343) may adhere badly to immuno sticks because of its polysaccharide capsule.

Apparently the 2 different panning approaches have different preferences in producing binders since no overlapping scFv antibodies were generated to the same target *B. mallei*. Both approaches can be used in complement to each other to obtain more scFv antibodies. In this study an approximately equal number of scFv antibodies were obtained against protein antigens (BM1, 1F3, and 3G5) and putatively conformational epitopes that are sensitive to SDS treatment (BP1, 2C5, 4F1, and 4G10). In a different study on panning a scFv library against *Helicobacter pylori* whole bacterial cells, Reiche *et al*. (16) obtained 2 scFv binders that recognized only native antigens. Our results also support that panning the scFv library against whole bacterial cells has a higher tendency to produce binders that recognize conformational epitopes or the native form of antigens than hyperimmunization in animals.

In conclusion, we have established alternative approaches for screening phage antibody libraries against surface antigens on heat-killed whole cells of highly pathogenic bacteria. A panel of human scFv antibodies that are potentially useful for diagnostic and therapeutic purposes against *B. mallei* and *B. pseudomallei* were obtained. The scFvs obtained in this study will be finally tested for applications in clinical diagnosis and/or treatment of *B. mallei* or *B. pseudomallei* infections. We are now in the process of restoring the scFvs to full human IgG format by fusing the heavy and light chain variable regions with the human γ- and κ-chain constant regions, respectively, and expressing them in cultured cells. Functional studies such as bacteria-neutralizing ability will be performed with the full-format antibodies. We are now also in the process of further characterizing the epitopes recognized by the obtained scFv phage antibodies, especially those recognized by BM1, which may be a dominant antigen epitope common to many Burkholderia species. This unique epitope(s) may prove to be highly valuable in the diagnosis and/or treatment of Burkholderia bacterial infections.

We thank Dr. Kim D. Janda of Scripts Institute in La Jolla, California, for kindly providing the human scFv library.

1. Anuntagool N, Sirisinha S. Antigenic relatedness between Burkholderia pseudomallei and Burkholderia mallei. Microbiol Immunol 46:143–150, 2002.

2. Leelarasamee A. Recent development in melioidosis. Curr Opin Infect Dis 17:131–136, 2004.
3. Woods DE, DeShazer D, Moore RA, Brett PJ, Burtnick MN, Reckseidler SL, Senkiw MD. Current studies on the pathogenesis of melioidosis. Microbes Infect 1:157–162, 1999.
4. Jeddeloh LA, Fritz DL, Waag DM, Hartings JM, Andrews GP. Biodefense-driven murine model of pneumonic melioidosis. Infect Immun 71:584–587, 2003.
5. Jones SM, Ellis JF, Russell P, Griffin KF, Oyston PC. Passive protection against Burkholderia pseudomallei infection in mice by monoclonal antibodies against capsular polysaccharide, lipopolysaccharide or proteins. J Med Microbiol 51:1055–1062, 2002.
6. Ho M, Schollaardt T, Smith MD, Perry MB, Brett PJ, Chaowagul W, Bryan LE. Specificity and functional activity of anti-Burkholderia pseudomallei polysaccharide antibodies. Infect Immun 65:3648–3653, 1997.
7. Pongsunk S, Thirawattanasuk N, Piyasangthong N, Ekpo P. Rapid identification of Burkholderia pseudomallei in blood cultures by a monoclonal antibody assay. J Clin Microbiol 37:3662–3667, 1999.
8. Steinmetz I, Reganzerowski A, Brenneke B, Haussler S, Simpson A, White NJ. Rapid identification of Burkholderia pseudomallei by latex agglutination based on an exopolysaccharide-specific monoclonal antibody. J Clin Microbiol 37:225–228, 1999.
9. Hoogenboom HR. Overview of antibody phage-display technology and its application. In: O'Brien PM, Aitken R, Eds. Antibody Phage Display: Methods and protocols. Totowa, NJ: Humana Press, pp. 1–37, 2002.
10. Rader C, Barbas CF 3rd. Phage display of combinatorial antibody libraries. Curr Opin Biotechnol 8:503–508, 1997.
11. McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. Nature 348: 552–554, 1990.
12. Lennard S. Standard protocols for the construction of scFv libraries. In: O'Brien PM, Aitken R, Eds. Antibody Phage Display: Methods and protocols. Totowa, NJ: Humana Press, pp. 59–71, 2002.
13. Nathan S, Li H, Mohamed R, Embi N. Phage display of recombinant antibodies toward Burkholderia pseudomallei exotoxin. J Biochem Mol Biol Biophys 6:45–53, 2002.
14. Gao C, Mao S, Kaufmann G, Wirsching P, Lerner RA, Janda KD. A method for the generation of combinatorial antibody libraries using pIX phage display. Proc Natl Acad Sci U S A 99:12612–12616, 2002.
15. Paoli GC, Chen CY, Brewster JD. Single-chain Fv antibody with specificity for Listeria monocytogenes. J Immunol Methods 289:147–155, 2004.
16. Reiche N, Jung A, Brabletz T, Vater T, Kirchner T, Faller G. Generation and characterization of human monoclonal scFv antibodies against Helicobacter pylori antigens. Infect Immun 70:4158–4164, 2002.
17. Stacy JE, Kausmally L, Simonsen B, Nordgard SH, Alsoe L, Michaelsen TE, Brekke OH. Direct isolation of recombinant human antibodies against group B Neisseria meningitidis from scFv expression libraries. J Immunol Methods 283:247–259, 2003.
18. Zhou B, Wirsching P, Janda KD. Human antibodies against spores of the genus Bacillus: a model study for detection of and protection against anthrax and the bioterrorist threat. Proc Natl Acad Sci U S A 99: 5241–5246, 2002.