Neutralization of *Burkholderia pseudomallei* Protease by Fabs Generated through Phage Display

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Received May 16, 2005; Accepted August 22, 2005

The isolation of therapeutic and functional protease inhibitors *in vitro* via combinatorial chemistry and phage display technology has been described previously. Here we report the construction of a combinatorial mouse-human chimeric antibody fragment (Fab) antibody library targeted against the protease of the tropical pathogen, *Burkholderia pseudomallei*. The resulting library was biopanned against the protease, and selected clones were analyzed for their ability to function as protease inhibitors. Three families of Fabs were identified by restriction fingerprinting, all of which demonstrated high specificity towards the protease of *B. pseudomallei*. Purified Fabs also demonstrated the capacity to inhibit *B. pseudomallei* protease activity *in vitro*, and this inhibitory property was exclusive to the pathogenic protease. Thus these recombinant antibodies are candidates for immunotherapy and tools to aid in further elucidation of the mechanism of action of the *B. pseudomallei* protease.

Key words: *Burkholderia pseudomallei*; Fab; phage display; protease

Proteases are directly or indirectly involved in virtually every biological function within cells. Accordingly, proteases and their naturally occurring macromolecular inhibitors are among the most extensively studied proteins in all of biochemistry.1) The catalytic mechanisms and substrate recognition properties of many proteases have been described, and distinct inhibitors have been developed for each protease class. This wealth of information makes these proteins ideal models for the investigation of structure-function relationships.1) Protease inhibitors have been prepared by structure-based design, combinatorial chemical synthesis, and phage display selection technologies.2) The selection of antibodies from combinatorial libraries displayed on the surface of filamentous phage has become important for the generation of reagent, diagnostic, and therapeutic molecules and for the study of the natural immune responses.3) Combinatorial antibodies are generated through the random recombination of cloned antibody heavy and light-chain fragments. When such libraries are cloned into phage display vectors, libraries of phages displaying unique antibody combinations on their surfaces can be selected efficiently. The inherent beauty of this whole system is the direct link between the phenotype displayed on the surface of the phage with the genotype encoding the displayed molecule found within the phage coat.4)

*Burkholderia pseudomallei* is an opportunistic pathogenic bacterium that secretes various extracellular virulent factors in the pathogenesis of melioidosis, a predominantly tropical disease that afflicts humans and animals.5) Although *B. pseudomallei* isolates are capable of expressing an impressive array of both secreted and cell-associated antigens, the role(s) of these products in the pathogenesis of the disease remain relatively ill-defined.6) Proteases, lecithinases, lipases, exotoxins, and hemolysins are the predominant secreted antigens that demonstrate biological activity.7–9) Cell-associated antigens described to date include acid phosphatase10) and capsular polysaccharides.11) The involvement of *B. pseudomallei* metalloprotease in the disease pathogenesis has been fairly well studied, and it has also been postulated that the importance of the protease as the immunodominant factor might surpass that of the exotoxin.9) Currently, no licensed vaccine preparation exists for immunization against melioidosis,6) and while one way of circumventing this shortfall is to identify and characterize antigens expressed by the pathogen to develop vaccine constructs, the use of antibodies for passive immunization is a viable alternative. In concert with the new era of design technology, the objective of this project was to utilize phage display technology to construct a combinatorial antibody library targeted...
against the protease of \textit{B. pseudomallei}. These antibodies might potentially serve as anti-protease molecules as well as concomitantly contributing to understanding of the structure-function relationships between the proteases. This paper reports on the construction and analysis of the recombinant mouse-human Fab clones and their specificity towards the \textit{B. pseudomallei} metalloprotease. Chimeric Fabs with human constant regions can easily be detected with anti-human Fab reagents, and they are usually better expressed in the bacterial host than are mouse antibody fragments.\textsuperscript{4} Mouse-human chimeric Fabs towards the \textit{B. pseudomallei} protease can also readily be channeled into the humanization of clones with therapeutic potential.

**Materials and Methods**

\textbf{Immunization of animals with \textit{B. pseudomallei} protease.} Protease was purified from 7-d stationary cultures of a human clinical isolate of \textit{B. pseudomallei} by ammonium sulfate precipitation followed by DEAE Cellulose and CM-Sepharose column chromatography, as previously described.\textsuperscript{11} The purified protease was subsequently used for the immunization of four Balb/C mice (henceforth referred to as \#1, \#2, \#3, and \#4) according to standard immunization procedures.\textsuperscript{12} The animals were given three boosts of the antigen and at appropriate times were bled, and sera samples were analyzed by indirect Enzyme-linked Immunosorbent Assay (ELISA) to determine titers. A 96-well plate (COSTAR, U.S.A.) was coated with 100 ng protease (in 0.1 m sodium bicarbonate (NaHCO\textsubscript{3}) buffer, pH 9.6) or with 1% BSA overnight at 4°C. The following day the wells were washed with distilled water and blocked with 5% milk powder (150μl) for 1 h at 37°C. Diluted sera (1:100) were added to the appropriate wells and incubated at 37°C for 2 h. Following extensive washing, alkaline phosphatase-conjugated goat anti-mouse IgG (1:1000, Pierce, U.S.A.) was added for 1 h at 37°C. Following extensive washing, the substrate was subsequently added, and color development was monitored at 405 nm. The following demonstration of sufficient antibody titer, the animals were given a final boost with the antigen, and 5 d later, the spleens were harvested and the mice were exsanguinated. Sera were obtained from the blood and stored at −20°C until required.

\textbf{Chimeric human-mouse Fab library construction.} Total RNA was prepared from harvested mice spleen using TRI Reagent (MRC, Cincinnati, OH) according to the manufacturer’s instructions. Briefly, spleens were homogenized and extracted RNA was precipitated with RNase-free isopropanol and dissolved in nuclease-free water. If necessary, the total RNA was further purified with lithium chloride (2 m), followed by precipitation with ethanol (100%) and sodium acetate (3 m) to improve the quality of the RNA. mRNA was reverse-transcribed with the SUPERSCRIPT Preamplification System for First Strand cDNA Synthesis Kit (Life Technologies, NJ) according to the manufacturer’s protocol. Typically, 20μg total RNA was utilized as template for priming by oligo-dT primers. Resulting first-strand cDNAs from each mouse were PCR amplified using mixes of 17 κ sense primers (Vκ-specific) and 4 κ anti-sense primers (Jκ-specific) for PCR amplification of the Vκ region. Only one sense and one anti-sense primer were used for amplification of the Vλ regions.\textsuperscript{4} Nineteen sense primers and three anti-sense primers were used for amplification of the VH regions. The kappa and lambda products were pooled, as were heavy-chain products, and purified using the Qiagen PCR Purification System or gel purified (Qiagen Gel Extraction System, Germany). The antisense primers consist of a hybrid mouse/human sequence designed for fusion of mouse V\textsubscript{H} and V\textsubscript{\lambda} coding sequences to human C\textsubscript{\lambda} and C\textsubscript{\gamma}1 coding sequences respectively. Human C\textsubscript{\lambda} and C\textsubscript{\gamma}1 coding sequences were amplified from a pComb3H-compatible expression vector\textsuperscript{3} that contained the sequence of a human Fab directed to tetanus toxoid\textsuperscript{13} using the primer combinations HKC-F (5-actgtggctgcacccatgtc-3')/lead-B (5-gccatggctggtggcgc-3') and HlgGCH1-F (5'-gctgcccaacgctgctggcagt-3')/dpseq (5'-agaagctgtggcgcgcagt-3') respectively. The anti-sense primer lead-B hybridizes to a sequence upstream of the Fd fragment coding sequence and is used to amplify the C\textsubscript{\lambda} coding sequence together with the sequence intervening light- and heavy-chain fragment coding sequences in phagemid vector pComb3H.\textsuperscript{14} Based on this strategy, the chimeric mouse/human light- and heavy-chain fragment coding sequences were assembled and fused by two sequential overlap extension PCR steps. In the first step, mouse V\textsubscript{H} and human C\textsubscript{\lambda} were fused using the primer combination RSC-F (5'-cgagtcggaggggaggggagggcctgcgctgctgctggcagyctggcagcagt-3')/lead-B, and mouse V\textsubscript{\lambda} and human C\textsubscript{\gamma}1 were fused using the primer combination lead-V\textsubscript{\lambda} (5-gctgcccaacgcgacgcagcagt-3') and dp-EX (5'-cgagtcggaggggaggggagggcctgcgctgctgctgcagtggcagcagt-3'). In the second step, the assembled chimeric light- and heavy-chain fragment coding sequences were fused by using the flanking primers RSC-F and dp-EX. Only light- and heavy-chain fragment coding sequences derived from the same animal were combined.\textsuperscript{14} Fab fragments of 1.5 kb were gel purified and cut with Sfi I (20 U/μg DNA, 5 h at 50°C and precipitated). The digested DNA was gel purified and used in a test ligation as follows: 70 ng of DNA was ligated into 140 ng of Sfi I cut pComb3H vector (gel purified) with 1U Ligase (Life Technologies, NJ) in a 20 μl reaction overnight at room temperature. One μl of the ligated product was transformed into electrocompetent ER2537 cells (New England Biolabs, U.S.A.) by electroporation to estimate the library size. For a library ligation, the reaction was scaled up by a factor of 10 under similar conditions. The DNA was precipitated and transformed into ER 2537 cells (see below). Two or 3
library ligations were performed to increase the complexity of the library. Fab constructs were also assembled by simultaneous assembly of 4-fragment overlap utilizing the primer combination of RSC-F and dp-EX, and the 1.5 kb product was treated as before.

**Library panning.** Ligated products were transformed into 300 μl ER 2537 cells (competency of at least 4 x 10^9–2 x 10^10 cfu/μg plasmid DNA) by electroporation (2.5 kV, 200 ohms, 25 μF), resulting in a complexity of 6 x 10^9 independent transformants. Four rounds of plate panning were performed against immobilized protease (1 μg in 25 μl 0.1 M bicarbonate buffer, pH 9.6 per well of a Costar #3690 96-well plate) and 5X PEG8000/NaCl precipitated phages were incubated on the protease coated wells for 2 h at 37°C. Unbound phages were washed 5, 10, 10, and 15 times with TBST (0.05%) for rounds 1, 2, 3, and 4 respectively. Bound phages were eluted by trypsinization (10 mg/ml, 30 min at 37°C) and used to infect log phase ER2537 cells. Amplification procedures with VCSM13 helper phage were followed as previously reported, while input and output phages were titrated on LB-Carb plates.

**Phage ELISA.** The original (unpanned) library and the output phage pool from each subsequent round of panning were analyzed by phage ELISA. Wells were coated with 1 μg protease, and 50 μl phage preparations were added and incubated for 2 h at 37°C. Following extensive washing, HRP-conjugated anti-M13 secondary antibodies (Amersham Pharmacia, U.S.A., 1:2000) were added. After a further incubation of 1 h at 37°C, ABTS–H_2O_2 substrate was added and color development was monitored at 405 nm.

**Single-colony phage and antibody (Fab) ELISA.** Forty clones from the final output plates of the panned libraries were selected and grown for 5 h in SB and carbenicillin (20 μg/ml). Antibody expression was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) (final concentration of 0.5 mM), and growth was continued overnight while cultures for phage production were grown in the presence of VCSM13 and antibiotics (carbenicillin and kanamycin) overnight at 37°C. Following centrifugation, the supernatants from both types of cultures were transferred to protease-coated wells and incubated for 2 h at 37°C. Detection of phages by anti-M13 antibodies was performed as described above. For Fab detection, 50 μl alkaline phosphatase conjugated anti-human Fab (1:1000 in milk, Pierce, U.S.A.) was added and incubated for 1 h at 37°C. Alkaline phosphate (50 μl) in developing buffer was added to the pre-washed wells and, absorption was read at 405 nm.

**Bst OI fingerprinting.** Phage DNA was prepared from the above selected clones from the final output plates of the panned libraries following the Qiagen Miniprep Kit protocol. Fab coding sequences were amplified using the ompseq (5'-aagacagctatgcagttg-3') and gback (5'-gcccccttattagctttgccatc-3') primer combination. The amplified products were subsequently digested with the 4 bp cutter Bst OI (Promega, U.S.A., 10–15 U) for 2 h at 60°C, and the fingerprints were analyzed on 3% agarose.

**Sequenceing.** Fab inserts were sequenced by automated cycle sequencing using the ompseq (5'-aagacagctatgcagttg-3') and newpelseq (5'-cattgctacgcacgccgtg-3') primers specific for the variable domains of the light- and heavy-chain respectively. DNA nucleotide sequences were translated into amino acid sequences using the DNA translator shareware at www.expasy.ch.

**Inhibition of protease activity.** Inhibition of B. pseudomallei protease activity was performed according to Percheron et al., with modifications. Selected clones were cultured in SB and carbenicillin (20 μg/ml), and antibody expression was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) (final concentration 0.5 mM). Antibodies were harvested and added to 1 μg protease and incubated for 30 min at 37°C. Azocasein (2%) and Tris–HCl (87.5 mM) were added to the incubated mix and further incubated for 5-min intervals up to 30 min. The reactivity was terminated with the addition of trichloroacetic acid and centrifuged (5 min/9,000 g). The supernatant (120 μl) was added to a microtiter plate well and incubated with 1 N NaOH, briefly followed by measuring absorbency at 405 nm. Commercial proteases and protease inhibitors were utilized as controls.

**Results**

**Sera antibody ELISA**

Antibody titers for mice immunized with the protease were monitored by ELISA. Following the final boost, the mice were exsanguinated and sera samples were analyzed against the protease (P) and also protease that was denatured by heat-treatment (DP), while BSA coated wells served as the negative control. DP antigens were tested due to the observation that Fab-bearing phagemid were selective towards denatured protease (see below). All four mice (#1–#4) indicated much better binding to the denatured protease (DP) than to non-heat treated protease (P), with the highest antibody response shown by mice #3 and #4 (Fig. 1). Hence, libraries were constructed from cDNAs prepared from mice #3 and #4.

**Library construction, panning, and analysis**

A mouse antibody library displayed on phages was generated as follows: RNA was isolated from bone marrow and spleen of the immune mice and, retro-transcribed, and V_L and V_H coding sequences were
amplified using a variety of primer combinations designed to amplify most of the known mouse antibody sequences. These primers were adapted for the pComb3H vector. Importantly, the mouse antibody library was based on a chimeric Fab format. Variable domains from mouse light- and heavy-chains were fused to corresponding human constant domains (Fig. 2). The human constant domains confer established and standardized detection and purification means of Fab derived from multiple species as well as improving the E. coli expression level of Fab. Therapeutically, chimeric mouse/human Fab can readily be channeled into previously reported strategies for complete humanization.

The phage library displaying chimeric mouse/human Fab was panned against immobilized B. pseudomallei protease. Two different mouse Fab libraries were used in the panning, and the size for both mouse libraries was estimated at approximately $3.9 \times 10^8$ cfu. Phage input for all libraries during panning was maintained at 1–2 x $10^{12}$ cfu. Output numbers for phage panned against protease increased in the second round but decreased significantly in the third round, and the low output numbers were maintained in the final round (data not shown). The drop in output numbers as well as the general lack of enrichment as observed in the corresponding phage ELISA (data not shown) was unexpected and might be a result of proteolytic activity of the immobilized B. pseudomallei protease. Markland et al. have noted that it was important to determine whether the target protease had an adverse effect on the viability of the bacteriophage since this usually results in a reduction of infectivity over a period of time due to the proteolytic degradation of the phage coat, particularly the gene III product. Alternatively, the protease might cleave off the phage-displayed antibody during the incubation period.

To overcome this problem, the protease was heat treated to 95°C prior to panning and both mouse libraries were re-panned against the native (non-heated)
protease (P), while one library was simultaneously panned against heat-treated (denatured) protease (DP). For the mouse library panned against P (mP) and DP (mDP), output numbers increased with more stringent washing over the four rounds of panning, indicating selection and enrichment of protease-specific binders (Fig. 3A). Nevertheless, the library panned against DP (mDP) exhibited much higher output numbers, leading to an enrichment of 38-fold for antigen specific Fab clones as compared to mP, suggesting that the Fab clones demonstrated more specific binding towards the denatured protease. This suggestion was further supported by the pooled phage ELISA, where phages harvested after each round of panning were incubated with native (P) and denatured (DP) protease. Figure 3B shows very little enrichment over the four rounds for the library panned against P in the ELISA when added to DP-coated wells, as compared to the BSA control, but the library that was panned against DP (mDP) had a strong enrichment of phage carrying DP specific antibodies. This indicates that during the immunization procedure, the protease was most likely denatured or underwent auto-proteolysis, and thus the mouse developed a stronger immune response towards the denatured form of the antigen than towards the native form (P). Furthermore, based on the stronger mouse sera ELISA response towards DP (see above), it can be speculated that the number of denatured protease molecules present following immunization was higher than that of the native antigen. When and how the antigen was denatured following immunization remains to be elucidated, and this information might serve to shed some light on the possible defense mechanisms applied by the infected host to alleviate pathogenesis by the protease antigen.

<table>
<thead>
<tr>
<th>Round of panning</th>
<th>Input (cfu/ml)</th>
<th>Output DP (cfu/ml)</th>
<th>Output P (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$10^{12}$</td>
<td>$6 \times 10^5$</td>
<td>$4.8 \times 10^5$</td>
</tr>
<tr>
<td>2</td>
<td>$10^{12}$</td>
<td>$4.6 \times 10^5$</td>
<td>$3.7 \times 10^5$</td>
</tr>
<tr>
<td>3</td>
<td>$10^{12}$</td>
<td>$180 \times 10^5$</td>
<td>$35 \times 10^5$</td>
</tr>
<tr>
<td>4</td>
<td>$10^{12}$</td>
<td>$230 \times 10^5$</td>
<td>$120 \times 10^5$</td>
</tr>
</tbody>
</table>

Fig. 3. Phage Output and ELISA after Four Rounds of Panning. A, Output of phage over four rounds of panning against DP and P indicating an enrichment of 38- and 25-fold respectively. B, Phage ELISA of DP-coating antigen towards amplified phage resulting from panning against □ DP and ■ P. ◼ BSA was used as the control.
Analysis of mDP-positive clones

Forty mDP-positive clones were selected and grown individually for simultaneous phage ELISA, antibody (Fab) ELISA (induced with IPTG), and fingerprinting analysis. The Bst OI fingerprint analysis on the 40 clones demonstrated three groups of highly repetitive patterns whereby groups I and II contained 10 identical members each, while group III was comprised of 7 members and the remaining 13 clones (group IV) were dissimilar but related (Fig. 4A). Of the 40 clones selected, 32 showed a strong response by antibody ELISA, indicating the presence of DP specific antibodies, while a further 3 clones had a weaker response. Figure 4b is a representation of a Fab antibody ELISA on selected clones (c19 [group III], c37 [group II] and c39 [group I]) that demonstrated a strong response to DP. The inserts of individual clones selected from the three antibody groups identified by fingerprint analysis were sequenced (Fig. 5). Sequences were obtained for the VH and the VL region, and all VH and VL sequences were identical to each other. When compared to the Kabat’s Database on Proteins of Immunological Interest, the VH sequence demonstrated 91% identity with mouse VH and 85% identity with human VH reported sequences, as expected.

The expressed Fabs from five clones (C9, C19, C32, C37, and C39) were tested for their ability to inhibit native B. pseudomallei protease activity (Table 1). These particular clones were selected based on their high absorbance values for the Fab ELISA. Fabs expressed from C19, C32, C37, and C39 were able to inhibit 62% to 82% of the original protease activity. Similar degrees of inhibition were also observed for protease treated with commercial metalloprotease inhibitors, EDTA and EGTA, while PMSF, a serine protease inhibitor, reduced protease activity by 50%. Concomitantly, the Fabs were also incubated with commercial proteases, and data presented in Table 1 demonstrate the selectivity of the Fabs to B. pseudomallei protease alone. The commercial proteases (trypsin and papain) were not inhibited by the Fabs, in contrast to their selective inhibition by their respective commercial inhibitors (PMSF and E64).
Phage display technology for antibodies and peptides has proven to be a highly effective method for finding needles in the molecular haystack. Various genetic approaches have been devised to capture the vast immunological repertoire and to generate and over-express antibodies. In phage antibody display, each phage particle contains an antibody gene fused to the gene for a phage coat protein, which results in the antibodies encoded by this gene, being displayed on the surface of the phage. This linkage between genotype and phenotype allows for very rare antibodies displayed on the phage to be selected from large repertoires of antibody variable region (V) genes by multiple rounds of affinity purification on antigen, a process referred to as affinity maturation.

Fig. 5. Amino Acid Sequence Alignment of a Representative Mouse Fab Clone 19 (FABc19), Fab Clone37 (FABc37), Fab Clone39 (FABc39), Mouse IgG-Kappa (Accession Nos J04438, M34588, L14370, Z22035, S40881 and Z22137) and Mouse IgG-Heavy (Accession Nos M98041, S67945, Z22076, P01801 and P01796) or Human IgG-Heavy Chain (Accession No G29380).

CDRs and FRs represent the sequences for complementarity determining regions and framework regions respectively for VH and VL regions. – indicates similarity between the Fab sequence and reported mouse/human immunoglobulin sequences.

Discussion

Phage display technology for antibodies and peptides has proven to be a highly effective method for finding needles in the molecular haystack. Various genetic approaches have been devised to capture the vast immunological repertoire and to generate and over-express antibodies. In phage antibody display, each phage particle contains an antibody gene fused to the gene for a phage coat protein, which results in the antibodies encoded by this gene, being displayed on the surface of the phage. This linkage between genotype and phenotype allows for very rare antibodies displayed on the phage to be selected from large repertoires of antibody variable region (V) genes by multiple rounds of affinity purification on antigen, a process referred to as affinity maturation.
biopanning. The technology for antibody design has taken enormous strides forward, and new library-display and library-selection procedures have provided methods for the production of monoclonal antibodies. The phagemid pComb3HSS is designed such that the antibody variable-region genes can be cloned between the leader sequence ompA and the truncated phage M13 gene III. Bacterial signal sequences direct transport of the protein to the inner membrane/periplasm of E. coli, where the main pIII domain attaches the fusion protein to the tip of the assembling phage. The greater part of mature phages with the potential to bind antigen display one copy of the antibody Fab fusion protein and two to four copies of the native pIII protein, which mediate phage attachment. The Fab protein is transported into the periplasmic space, but is not assembled into a phage particle. Upon induction by isopropyl-D-thiogalactopyranoside (IPTG), the soluble Fab antibody accumulates in the periplasm, and following extended incubation leaks into the medium. The combination of pyranoside (IPTG), the soluble Fab antibody accumulates in the periplasm, and following extended incubation leaks into the medium. The combination of the protease as well as the serine protease secreeted by B. pseudomallei might contribute significantly to tissue damage as well as the ability for intracellular survival within the host for extended periods. Thus proteases are viable targets for developing inhibitors.

The role played by B. pseudomallei metalloprotease in the pathogenesis of melioidosis is not yet fully understood. The metalloprotease as well as the serine protease secreted by B. pseudomallei might contribute significantly to tissue damage as well as the ability for intracellular survival within the host for extended periods. Thus proteases are viable targets for developing inhibitors. These proteases are expected to target the substrates that are involved in the pathogenesis of melioidosis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Inhibition* of protease activity</th>
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<tbody>
<tr>
<td>PBS + protease</td>
<td>2</td>
</tr>
<tr>
<td>Mouse Sera + protease</td>
<td>87</td>
</tr>
<tr>
<td>Fab C9 + protease</td>
<td>33</td>
</tr>
<tr>
<td>Fab C19 + protease</td>
<td>65</td>
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<tr>
<td>Fab C32 + protease</td>
<td>64</td>
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<tr>
<td>Fab C39 + protease</td>
<td>82</td>
</tr>
<tr>
<td>Fab C37 + protease</td>
<td>62</td>
</tr>
<tr>
<td>EGTA (30 mM) + protease</td>
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<tr>
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<td>PMSF (1 mM) + protease</td>
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<tr>
<td>Tryptsin + PMSF</td>
<td>84</td>
</tr>
<tr>
<td>Tryptsin + Fab C39</td>
<td>22</td>
</tr>
<tr>
<td>Papain + E64</td>
<td>72</td>
</tr>
<tr>
<td>Papain + Fab C39</td>
<td>23</td>
</tr>
</tbody>
</table>

*Proteolytic activity (as measured by a standard azocasein assay) of B. pseudomallei protease (control) was taken to be 100% (or 0% inhibition). The proteolytic activity (% of control) of the protease in the presence of buffer (PBS), Fab, or commercial inhibitor was measured. The % inhibition was then calculated as (100% activity [control] – X% activity of protease + buffer/Fab/inhibitor).

Neutralization of Burkholderia pseudomallei Protease

Table 1. Inhibition of B. pseudomallei Protease Activity by Selected Fab Crude Samples

Mouse serum was used as a positive control, while EDTA, EGTA, and PMSF (Phenylmethylsulfonyl fluoride) are known protease inhibitors. A selected Fab and inhibitors were also tested against a commercial trypsin and pапain.

The proteolytic activity (% of control) of the protease in the presence of buffer (PBS), Fab, or commercial inhibitor was measured. The % inhibition was then calculated as (100% activity [control] – X% activity of protease + buffer/Fab/inhibitor).
years has led to a remarkable awareness of the pivotal role played by these molecules in every aspect of cellular and tissue function. The new era in design technology should see rational approaches to the construction of protease inhibitors that target in a highly specific manner.

Acknowledgments

We would like to acknowledge the helpful comments and stimulating advice of Dr. P. Steinberger (The Scripps Research Institute, TSRI). The assistance of Ms. K. Bower (TSRI) in animal immunizations and of Ms. S. W. Chan and Ms. C. S. Koh (CGAT, UKM) for technical assistance is appreciated. This project was supported by the Skaggs Institute for Chemical Biology and by the IRPA Top-Down Grant (Malaysia) 09-02-02-T001. S.N. was the recipient of a Fulbright Award from the Council for International Exchange of Scholars (U.S.A.). The antibody sequences presented in this study are deposited in the Genbank database under accession nos. AF411462-AF411463.

References

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