Filamentous Hemagglutinin Polyclonal Antibodies Protect Against Multidrug-Resistant Gram-Negative Bacteria

Eman Youssef,[1,2*] Sondus Alkhazraji,[1] Shakti Singh,[1] Teklegiorgis Gebremariam,[1] Ashraf S. Ibrahim.[1,3*]

[1]The Lundqusit Institute at Harbor-UCLA Med Ctr., Torrance, California; [2]Biotechnology Department, Beni-Suef University, Egypt; [3]David Geffen School of Medicine at UCLA, Los Angeles, California

Ibrahim@Lundquist.org
eman.youssef@Lundquist.org

INTRODUCTION

- Healthcare-associated infections due to MDR GNB such as *Acinetobacter baumannii* (AB), *Pseudomonas aeruginosa* (PA) and *Klebsiella pneumonia* (KP) are increasing in numbers and are associated with high mortality rates (1).
- New methods to prevent or treat these infections are needed.
- The *Candida albicans* (CA) antigen Hyr1p shares structural and sequence homology with the hemagglutinin/hemolysin protein (FhaB) of GNB including AB.
- Our studies showed that passive immunization targeting the CA Hyr1 peptide#5 are protective in AB pneumonia mouse model (2, 3).
- In this study, we identified FhaB epitopes that shared sequence homology with CA Hyr1 peptide#5.
- We aim to evaluate antibody-based therapy targeting these epitopes against AB and PA infection

METHODS

- Sequence homology between *Candida* Hyr1#5 and AB-FhaB protein was performed using BLAST, EMBOSS-Water, and EMBOSS-Needle online tools.
- Seven 14-mer peptides were selected for synthesis commercially and generation of rabbit polyclonal Abs.
- ***ELISA:*** A 96-well plate was coated with synthesized peptides for overnight at 4°C. Anti-Hyr1#5 Ab were used as 1° Ab and HRP-conjugated as 2° Ab.
- ***Flow cytometery:*** 100 µg/ml of FhaB Abs were incubated with $5x10^6$ log phase bacteria for 1 h and anti-FITC counter stain rabbit IgG used to stain cells.
- ***AB mouse model:*** CD-1 mice were immunosuppressed by cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3, and +8 relative to infection. Mice were infected with MDR HUMC1 strain by using areoslization chamber to induce pneumoniae.
- ***PA mouse model:*** CD-1 mice were immunosuppressed by giving one dose of cyclophosphamide (200 mg/kg) and cortisone acetate (250 mg/kg) at day -2 relative to infection. Mice were infected with MDR PA-01 strain intratracheally.
- For survival studies, 30 µg/mouse Abs or isotype matching control were administrated i.p. as a single dose 16 h post infection. Survival served as an endpoint.
- Statistical analysis was carried out by Log-rank Sum (Mantel-cox) test for the survival studies with *P* values of <0.05 being significant.

REFERENCES

1. Jones. *Clin Infect Dis*. 2010.
2. Uppuluri *et al. PLoS Pathogens* 2018
3. Youssef *et al. Front Immunol.* 2020.

Table (1): List of AB-FhaB peptides share sequence similarity to Hyr1#5. Seven peptides designed from AB-FhaB protein, showing sequence similarity to Hyr1#5.

Peptide sequence	Alignment to Hyr1#5	Percent similarity to Hyr1#5
>**FhaB#1** LENAATRVKNIKAY	FhaB_Epitope#1 LENAATRVKNIKAY Hyr1#5 LKNAVTYDGPVPNN	% Identity = 4/14 (~ 30%) % Similarity = **6/14** (~ 43%)
>**FhaB#2** TAQLPSFNGPVLPT	FhaB_Epitope#2 TAQLPSFNGPVLPT Hyr1#5 LKNAVTYDGPVPNN	% Identity = 3/14 (~ 21%) % Similarity = **7/14** (~ 50%)
>**FhaB#3** GSIAVGINSKSVGNN	FhaB_Epitope#3 GSIAVGINSKSVGNN Hyr1#5 LKNAVTYDG-PVPNN	% Identity = 5/14 (~ 36%) % Similarity = **6/14** (~ 43%)
>**FhaB#4** KQFDVLAEGAVLNN	FhaB_Epitope#4 KQFDVLAEGAVLNN Hyr1#5 LKNAVTYDGPVPNN	% Identity = 5/14 (~ 36%) % Similarity = **7/14** (~ 50%)
>**FhaB#5** VVNIQTPKNGISHN	FhaB_Epitope#5 VVNIQTPKNGISHN Hyr1#5 LKNAVTYDGPVPNN	% Identity = 3/14 (~ 21%) % Similarity = **6/14** (~ 43%)
>**FhaB#6** PKNGISHNIYKQFD	FhaB_Epitope#6 PKNGISHNIYKQFD Hyr1#5 LKNAVTYDGPVPNN	% Identity = 2/14 (~ 14%) % Similarity = **6/14** (~ 43%)
>**FhaB#7** MPLAPVYAGIVADS	FhaB_Epitope#7 MPLAPVYAGIVADS Hyr1#5 LKNAVTYDGPVPNN	% Identity = 4/14 (~ 30%) % Similarity = **6/14** (~ 43%)

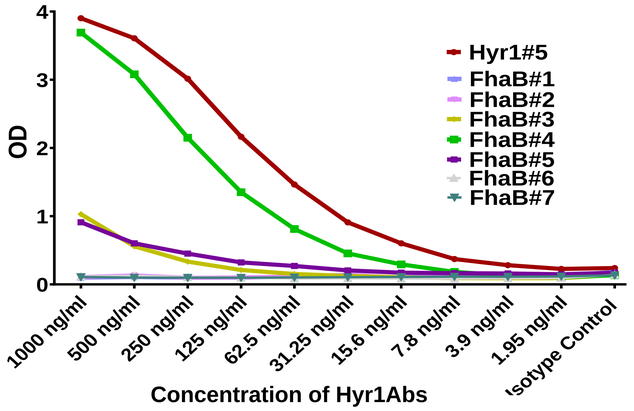

Figure 1. Recognition of synthesized FhaB peptides to Hyr1#5 Abs compared. FhaB#4 peptide showed strong binding to Hyr1#5 Abs comparable to Hyr1#5 peptide. However, FhaB#3 and FhaB#5 were of week binding.

ACKNOWLEDGEMENTS

This work is funded by NIAID R01AI141202

RESULTS

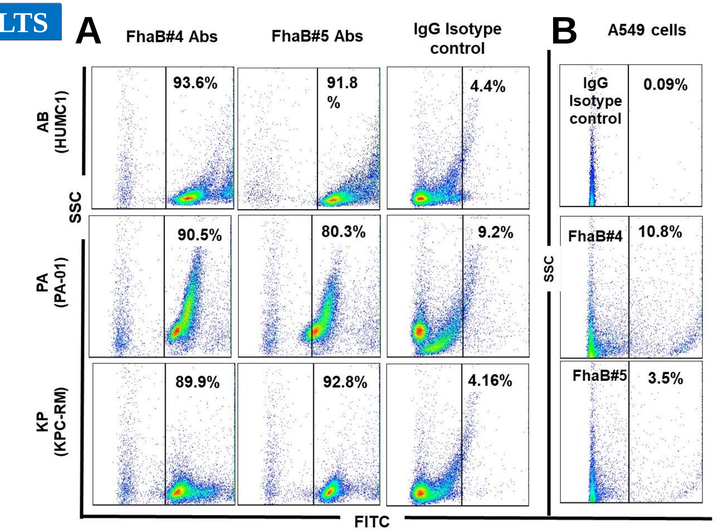

Figure 2. Binding profile of FhaB Abs. FhaB#4 & FhaB#5 pAbs bind to different MDR strains of GNB **(A)**. Both FhaB#4 & FhaB#5 pAbs didn't recognize lung epithelial cells (A549), supporting specificity of pAbs to bacteria rather than mammalian cells. **(B)**

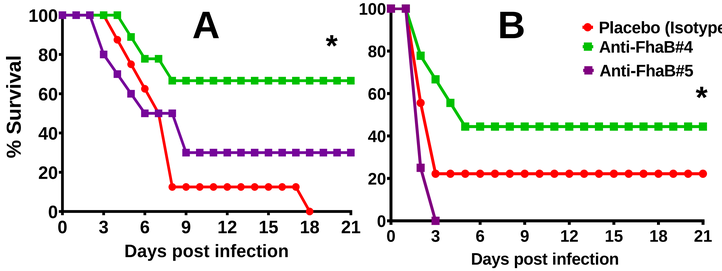

Figure 3. Combined survival data. Immunosuppressed mice infected with AB (HUMC-1) **(A)** or PA (PA-01) **(B)** treated with FhaB#4,FhaB#5 pAbs or isotype matching control at day +1**.** N= 10 per group. **P* < 0.05 vs. placebo.

SUMMARY/CONCLUSIONS

- *Candida* Hyr1#5 peptide shares ~50% homology with AB-FhaB.
- FhaB#3, FhaB#4 and FhaB#5 peptides reacted to Hyr1#5 antibodies.
- Anti-FhaB#4 and Anti-FhaB#5 pAbs bind to AB, PA, and KP.
- Passive immunization with anti-FhaB#4 protect mice from AB and PA pneumonia.
- These data support further development of these Abs as novel immunotherapeutics against MDR GNB.