Subject: NOTE NEW DATE: Dissertation Defense - Intisar Alruwaili, PHD Biosciences
Date: Wednesday, November 6, 2024 at 3:00:12 PM Eastern Standard Time
From: SSB Faculty List on behalf of Diane St. Germain
To: SSB-FACULTY-LIST-L@LISTSERV.GMU.EDU

Dissertation Defense Announcement
To: The George Mason University Community

Candidate: Intisar Alruwaili

Program: PhD in Biosciences

Date: Tuesday, November 19, 2024

Time: 12:00 p.m. EST

Location: Virtual via Microsoft Teams: Teams link
Meeting ID: 264 093 496 504
Passcode: vESzGY
Dial in by phone
+1 571-397-2084,,894874113# United States, Washington
Find a local number
Phone conference ID: 894 874 113#

Committee Chair: Dr. Monique van Hoek

Committee members: Dr. Urszula Krzych, Dr. Aarthi Narayanan, Dr. Alessandra Luchini

Title: Monoclonal Antibodies Directed Against Surface Antigens of *Klebsiella Pneumoniae* as Potential Immunoprophylactic Treatment against Bacterial Infection

Abstract:
Antimicrobial resistance represents a significant threat to global health, contributing to millions of deaths annually. *K. pneumoniae* is responsible for a wide range of infections, including pneumonia, bloodstream infections, and urinary tract infections, often leading to increased morbidity and mortality due to its resistance to multiple antibiotics. This critical global situation requires urgent need for alternative therapeutics, for example, immunotherapies based on highly effective monoclonal antibodies (mAbs). We hypothesize that mAbs targeting critical *K. pneumoniae* surface proteins, such as outer membrane proteins and siderophores, can serve as an effective prophylactic or therapeutic strategy against MDR infections. In this study, we developed murine mAbs against two key proteins of *K. pneumoniae*, outer membrane protein (OmpW) and the

siderophore receptor (FepA), both of which are associated with bacterial virulence and survival. We expressed both proteins in *E. coli* and used the recombinant proteins as adjuvanted immunogens for immunization and induction of activated B cells in BALB/c mice. We generated mAbs using standard hybridoma fusion techniques with SP2/0 myeloma cells. Supernatants from positive hybrids, as determined microscopically, were screened for antibody specificity using enzyme-linked immunosorbent assay (ELISA). Hybridomas that produced high titers of antibodies were cloned by limiting dilution to achieve monoclonal antibodies. Culture supernatants from the clonal population were screened again, and the developed *K. pneumoniae* mAbs were characterized for their class and isotype as well as their ability to bind to the immunogen and *K. pneumoniae*. mAbs of each specificity were also evaluated for their ability to engage in opsonophagocytosis (OP) using murine phagocytic macrophage cell lines, which express Fc receptors crucial for antibody-mediated clearance. The OP assay, based on flow cytometry, was optimized using fixed and pHrodo Red-labeled *K. pneumoniae* to quantify the phagocytic uptake by macrophages. Results demonstrated that mAbs targeting OmpW-2, but not OmpW-1, and FepA significantly increased OP, as determined by the mean fluorescence intensity, compared to the control mAbs. We also evaluated the OmpW and FepA mAbs for their efficacy to prevent *K. pneumoniae* infection using the *Galleria mellonella* worm model. Measuring the survival of *G. mellonella* larvae for either 24 or 48 hours in the presence of mAbs, we observed that while some mAbs were quite effective (e.g., anti-OmpW-1), mAbs targeting the other specificities, OmpW-2 and FepA, were ineffective. Overall, these data suggest that targeting surface proteins of *K. pneumoniae* with mAbs could enhance bacterial clearance in vitro and in vivo, although further studies are needed to optimize mAb dosage and improve synergistic effects for potential clinical applications.

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