How Antibody Isotype Affects Anti-Capsular Antibody Protection Against Carbapenem-Resistant Klebsiella pneumoniae Infection

Michael P Motley[1,2,3], Elizabeth Diago-Navarro[1], Kasturi Banerjee[1], Bettina C Fries[1,2]

[1]Department of Medicine, [2]Department of Molecular Genetics and Microbiology, and [3]Medical Scientist Training Program, Stony Brook University, Stony Brook, NY

Stony Brook Medicine

BACKGROUND

- Carbapenem-Resistant *Klebsiella pneumoniae* (CR-*Kp*) causes serious high-mortality infections.
- Monoclonal antibodies (mAbs) can be used to mediate disease, and our laboratory has developed the murine anti-capsular IgG_3 mAb 17H12 with *in vitro* and *in vivo* activity against a large subset of CR-*Kp* isolates.[1]
- Human and Murine IgG antibodies each have 4 different subclasses, which differ in their ability to activate or suppress immunity, promote phagocytosis, fix complement, and bind their desired antigen.[2]
- Our previous studies showed that an IgG_1 mAb performed better than an IgG_3 mAb in mediating infection against a carbapenem-sensitive *Kp* isolate.[3]

HYPOTHESIS

Isotype subclass variants of 17H12 will alter the efficacy of the antibody in mediating protection against Carbapenem-Resistant Klebsiella pneumoniae

METHODS

- 17H12 IgG_3 hybridomas were treated with LPS and IL-4 over one week to induce subclass switching.
- Spontaneous subclass recombinants were identified by ELIspot, and purified through sib selection, FACS, and soft-agar cloning
- New clones were sequenced and compared with the complementary-determining region (CDR) sequence of the IgG_3 for somatic mutations.
- Binding kinetics of the two mAbs were compared using ELISA against CR-*Kp* capsular polysaccharide.
- Opsonophagocytosis was assessed in J774.16 cells and human neutrophils by enumerating CFUs found within phagocytes after 30 min of incubation with CR-*Kp* strains pre-opsonized with IgG_1 or IgG_3 17H12. Assays were performed in 10% FBS for macrophages, or 20% fresh or heat-killed normal human serum for neutrophils.
- Complement deposition was assessed using flow cytometry to compare the relative fluorescence index of CR-*Kp* bacteria labeled with anti-C3C antibody after incubation with mAbs with or without NHS for 30min.
- BALB/c mice were infected intratracheally with $1x10^7$ CFU CR-*Kp* pre-opsonized with 20μg 17H12 IgG_1 or 17H12 IgG_3, or a control IgG_1. Mice were sacrificed after 24 hr and bacterial burden within the lung, liver, and spleen was enumerated by CFU counts

RESULTS

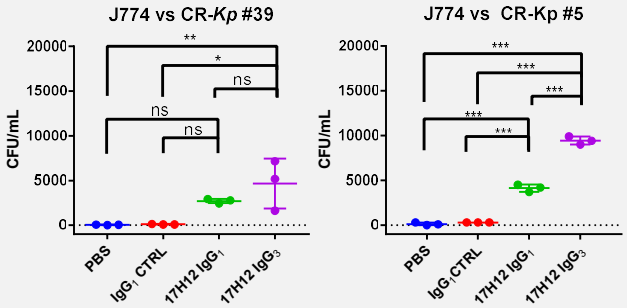
A. SEQUENCE CHARACTERISTICS (BOTH CDRs IDENTICAL)

mAb	V_H gene and family	J_H gene	D gene	V_L family	V_L gene	J_L gene
17H12	AJ851868 IGHV5-12*02	IGHJ2*03	IGHD4-1*01	Z72384	IGKV1-135*01	IGKJ1*01

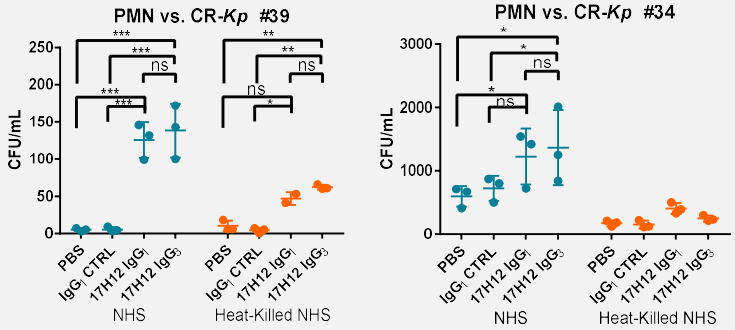
B. AFFINITIES (ELISA vs CPS)

mAB	17H12 IgG_1 (new)	17H12 IgG_3 (parent)
K_D	20.1 nM	2.2 nM

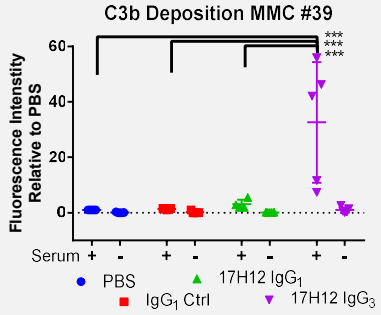
C. MACROPHAGE PHAGOCYTOSIS



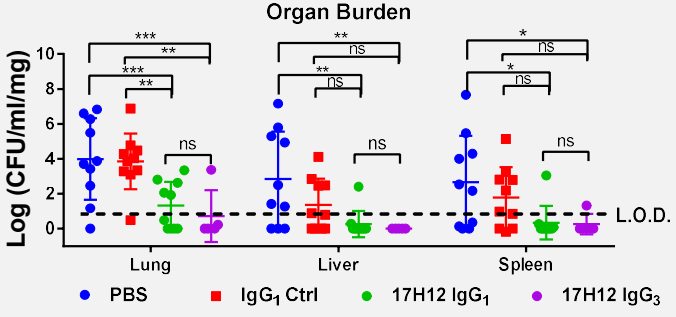
D. NEUTROPHIL PHAGOCYTOSIS



E. COMPLEMENT DEPOSITION



F. PROTECTION FROM INTRATRACHEAL INFECTION



CONCLUSIONS

- mAb IgG subclass alters binding efficacy and cooperation with the immune system, but showed limited differences *in vivo* against infection.
- Understanding differences in the interactions between the immune system and mAb of different isotypes and subclasses will help the development of mAb therapeutics against CR-*Kp*
- Future experiments will examine the differences in Fc Receptor Interactions and their ability to alter host-cytokine response to infection.

REFERENCES

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