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

Michael P Motley, Elizabeth Diago-Navarro, Kasturi Banerjee, Bettina C Fries

Department of Medicine, Department of Molecular Genetics and Microbiology, and Medical Scientist Training Program, Stony Brook University, Stony Brook, NY

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 IgG3 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 IgG3 mAb performed better than an IgG3 mAb in the mediation 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 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 IgG1 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 strain pre-opsonized with IgG3 or IgG1 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 IgG3 or 17H12 IgG1, or a control IgG1. Mice were sacrificed after 24 hr and bacterial burden in the lung, liver, and spleen was enumerated by CFU counts

RESULTS

A. SEQUENCE CHARACTERISTICS (BOTH CDRs IDENTICAL)

<table>
<thead>
<tr>
<th>mAb</th>
<th>Vι gene and family</th>
<th>Jι gene</th>
<th>D gene</th>
<th>Vι family</th>
<th>Vι gene</th>
<th>Jι gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>17H12 A/J851868</td>
<td>IGHV1-12*02</td>
<td>IGJH2*03</td>
<td>IGHD4-1*01</td>
<td>Z72384</td>
<td>IGVK1-135*01</td>
<td>IGLJ1*01</td>
</tr>
</tbody>
</table>

B. AFFINITIES (ELISA vs CPS)

<table>
<thead>
<tr>
<th>mAb</th>
<th>17H12 IgG3 (new)</th>
<th>17H12 IgG3 (parent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kd</td>
<td>20.1 nM</td>
<td>2.2 nM</td>
</tr>
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</table>

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

2. Collins AM. Immunology And Cell Biology 2016; 94:949.

ACKNOWLEDGEMENTS

Funding was provided by the NIH (R21-A114259) and Dr. Fries’ Stony Brook University Startup Fund. Authors have no financial conflicts of interest.