Help is on the way: Monoclonal antibody therapy for multi-drug resistant bacteria

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ARTICLE HISTORY Received 8 March 2017; Accepted 8 March 2017

KEYWORDS antibiotic resistance; drug resistant; Fc receptor; Gram negative organisms; Klebsiella pneumoniae; monoclonal antibody therapy

Antibiotic resistance is a substantial global threat to human health. Antibiotic use, overuse, and misuse has led to the emergence of multi-drug resistant bacterial strains that continue to present a major therapeutic challenge in the clinic. A recent mortality caused by a strain of Klebsiella pneumoniae that was resistant to all 26 antibiotics currently available to US clinicians exemplifies the urgent need for potent treatment modalities for drug resistant bacteria that do not increase the risk of antibiotic resistance. One promising alternative to antibiotics are pathogen-specific monoclonal antibodies (mAbs). The concept of mAb therapy originates from the successful development and use of serum therapy for bacterial infections. The efficacy of this modality, which consisted of specific antisera that were the inaugural antimicrobial agents, was validated in clinical trials and used in clinical practice from the early part of the 20th century until the 1940s. Serum therapy was abandoned with the arrival of antibiotics, in part due to numerous toxicities and the inability to purify or produce antibodies to single determinants at the time. However, today, advances in molecular biology, technology and antibody engineering make it possible to generate defined, homogenous, fully human and/or humanized mAbs with a single antigen-specificity to target a pathogen of interest. In fact, mAb generation only requires an antigen (immunogen) and an immune or immunized individual or immunization platform. To date, mouse models, as exemplified by the study of Szijártó and colleagues that served as the basis for their work reported in this issue of Virulence, are the most tried and true. Due to their potency, specificity, and safety profiles, mAbs have entered the oncology armamentarium and are increasingly used for treatment of rheumatologic and inflammatory diseases. Although at present, only one mAb, Palivizumab, for treatment of respiratory syncytial virus (RSV) in high risk infants, is licensed in the US, several candidate mAbs are now in advanced clinical trials for other infectious diseases such as HIV, Clostridium difficile, rabies prophylaxis and Staphylococcus aureus.

In this issue of Virulence, Szijártó and colleagues describe a candidate mAb for treatment of multi-drug resistant Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae. Globally, most KPC-produ
cing K. pneumoniae isolates are associated with the multilocus sequence type ST258. In previous work, the authors established that most of K. pneumoniae ST258 (hereafter, ST258) isolates express the LPS O-antigen, D-galactan-III (gal-III). Based on this finding, they generated gal-III-specific mouse mAbs with the hope of identifying mAbs that might serve as a therapeutic agent for the main ST258 clades. The current study reports the biologic activity in vitro and in vivo efficacy in experimental models of ST258 infection of A1102, a humanized mouse gal-III mAb that expresses human kappa and IgG1 constant regions. The data demonstrate that passive immunization with A1102 before lethal challenge with ST258 whole bacteria or ST258-derived LPS prolongs survival of endotoxin-sensitized mice and protects rabbits from a lethal ST258 challenge. In vitro studies show that the biologic activity of A1102 includes complement- and Fc-independent LPS neutralization that requires divalent binding, and enhancement of human serum bactericidal killing and complement-dependent macrophage (RAW267.4 cell) uptake of ST258.

Interestingly, the in vitro activity of A1102 does not provide a singular correlate of how it mediates protection in vivo. For instance, although it exhibits bactericidal and opsonic activity that require complement in vitro, A1102 and an aglycosylated IgG1 mutant derivative which cannot bind C1q were...
protective in cobra venom-treated and normal mice, respectively. Based on their findings that complement and C1q-mediated complement activation are dispensable for A1102 efficacy in vivo, the authors suggest that Fc-independent LPS neutralization may be the main mechanism by which A1102 mediates protection in vivo. However, the role that Fc receptors might play in A1102-mediated protection was not directly examined in this study. Of relevance to the question of how A1102 neutralizes LPS in vivo, mAb-mediated toxin neutralization can be Fc receptor dependent. In fact, although complement was unnecessary for the efficacy of a non-opsonic Streptococcus pneumoniae capsular polysaccharide-binding mAb in vivo, FcyRIIIA (and the mAb’s Fc) was required for protection against sepsis and pneumonia in mice. However, for other antibodies to S. pneumoniae, complement was required to promote bacterial uptake by phagocytes depending on the amount of antibody present. Antibodies are versatile molecules capable of multiple modes of action ranging from direct effects on the targeted microbe to toxin neutralization to enhancement of phagocytosis and immune modulation. Thus, experiments to evaluate the efficacy of whole A1102 versus its F(ab’)2 fragments in normal and Fc receptor-deficient mice could be used in the future to dissect the roles that Fc-dependent and Fc-independent activities play in A1102-mediated protection in vivo. Since studies of A1102 efficacy in mice feature a species mismatch between human IgG1 and mouse Fc receptors, use of mice expressing human Fc receptors could be considered.

Fcγ receptors regulate immune responses upon binding antigen-IgG complexes via an interplay of activating and inhibitory receptors. Different IgG subclasses have different affinities for Fc receptors that in concert can elicit or limit inflammation. Human IgG1, the isotype of A1102, binds activating Fc receptors more avidly than the inhibitory Fc receptor. Thus, the inhibitory Fc receptor might enhance A1102 efficacy by balancing or dampening inflammation. This was the case for an opsonic mAb to S. pneumoniae that required the inhibitory Fc receptor to mediate protection. On the other hand, if F(ab’)2 fragments of A1102 neutralize LPS in vivo, this might limit cytokine-mediated inflammation triggered by LPS and/or Fcγ receptor activation. However, notably, even though F(ab’)2 fragments of a S. pneumoniae capsule-specific mAb reduced nasopharyngeal colonization and prevented lung dissemination in mice, the whole mAb was required to protect against systemic infection and pneumonia and modulate inflammation in the nasopharynx. Along the same lines, F (ab’)2 fragments of polyclonal IgG provided optimal protection against colonization in another mouse model of S. pneumoniae colonization. In these examples, antibodies that were effective against nasopharyngeal colonization exhibited the ability to agglutinate bacteria. A similar result was obtained with human post-pneumococcal vaccine samples.

K. pneumoniae possesses a polysaccharide capsule, an important virulence factor and mechanism of antigenic variation. Thus, another possible therapeutic modality for K. pneumoniae infections are capsule-binding mAbs. Consistent with this concept, K1 capsular polysaccharide (CPS)-specific mouse IgG1 mAbs were protective in murine models of K. pneumoniae sepsis and pulmonary infection. These antibodies enhanced in vitro Fc receptor mediated phagocytosis of K. pneumoniae and modulated cytokine levels in vivo. However, a drawback of this approach is the heterogeneous nature of the K. pneumoniae CPS, which precludes development of mAbs that could be used irrespective of CPS type. Therefore, antibodies such as A1102 that target conserved antigens, e.g. the LPS O-antigen, may find a faster place in the immunotherapy armamentarium for K. pneumoniae. Nevertheless, use of a multitude of mAbs that target different determinants and work by different mechanisms may be required to protect against various K. pneumoniae strains and disease manifestations.

A major strength of mAb A1102 lies in its potency against experimental K. pneumoniae infection. In general, endotoxin-neutralizing antibodies have had relatively low affinity and historically were largely unsuccessful in the clinic. In contrast, A1102 exhibits high affinity, neutralizes LPS better than polymyxin B in vitro, and doses as low as 3 μg for mice and 2 μg/kg for rabbits mediate protection in vivo. The ability to achieve protection with small doses could significantly lower the cost of treatment, highlighting the promising potential of A1102 for therapy of K. pneumoniae infections. However, one caveat is that the current study shows A1102 mediates protection when given before infection. Although high risk patients might be candidates for preemptive or prophylactic mAb therapy, further work is needed to establish the potency of A1102 as a therapeutic agent when infection has already occurred. Nonetheless, in the serum therapy era, sera that were protective in experimental models when given before infection were protective in patients presenting with symptoms, though sera had to be given early in the course of disease to be effective. In this regard, the revolution in rapid diagnostics that is making pathogen identification possible within hours will enhance the feasibility of mAb-based therapy for K. pneumoniae and other pathogens.
Although ST258 is the most predominant multi-drug resistant *K. pneumoniae* sequence type, one question raised by this study is how effective A1102 will be against other sequence types. Nevertheless, the data in this study is how effective A1102 will be against *K. pneumoniae* resistant indicative that A1102 is a potent mAb that holds significant promise as a therapeutic agent for multi-drug resistant *K. pneumoniae* which could help stem the rising tide of antibiotic resistance.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Funding**

Funding was provided by NIH grants R01AI123654 and R01AG045044 to LP.

**References**

[1] Chen L. Notes from the Field: Pan-Resistant New Delhi Metallo-Beta-Lactamase-Producing Klebsiella pneumoniae—Washoe County, Nevada, 2016. MMWR Morb Mortal Wkly Rep 2017; 66(1):33; PMID:28081065; https://doi.org/10.15585/mmwr.mm6601a7

[2] Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in infectious diseases. Clin Infect Dis 1995; 21(1):150-61; PMID:7578724; https://doi.org/10.1093/clinids/21.1.150

[3] Jolliffe LK. Humanized antibodies: enhancing therapeutic utility through antibody engineering. Int Rev Immunol 1993; 10(2–3):241-50; PMID:8360588; https://doi.org/10.3109/08830189309061699

[4] Szijarto V, Guachalla LM, Hartl K, Varga C, Badarau A, Mirkina I, Visram ZC, Stulik L, Power CA, Nagy E, et al. Endotoxin neutralization by an O-antigen specific monoclonal antibody: a potential novel therapeutical approach against Klebsiella pneumoniae ST258. Virulence 2017; 1-13; PMID:28103139; https://doi.org/10.1080/21505594.1.1279778

[5] Kotsovillis S, Andrekos E. Therapeutic human monoclonal antibodies in inflammatory diseases. Methods Mol Biol 2014; 1060:37-59; PMID:24037835; https://doi.org/10.1007/978-1-62703-586-3_3

[6] Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer 2012; 12(4):278-87; PMID:22437872; https://doi.org/10.1038/nrc3236

[7] Johnson S, Oliver C, Prince GA, Hemming VG, Pfarr DS, Wang SC, Dormitzer M, O’Grady J, Koenig S, Tamura JK, et al. Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. J Infect Dis. 1997; 176(5):1215-24; PMID:9359721; https://doi.org/10.1086/314115

[8] Bakker AB, Python C, Kissling CJ, Pandya P, Marissen WE, Brink MF, Lagerwerf F, Worst S, van Corven E, Kostense S, et al. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. Vaccine 2008; 26 (47):5922-7; PMID:18804136; https://doi.org/10.1016/j.vaccine.2008.08.050

[9] Lowy I, Molrine DC, Leav BA, Blair BM, Baxter R, Gerding DN, et al. Treatment with monoclonal antibodies against Clostridium difficile toxins. N Engl J Med 2010; 362(3):197-205; PMID:20089970; https://doi.org/10.1056/NEJMoa0907635

[10] Hsu L, Hilliard JJ, Shi Y, Tkaczyk C, Cheng LI, Yu X, Datta V, Ren S, Feng H, Zinsou R, et al. Assessment of an anti-alpha-toxin monoclonal antibody for prevention and treatment of Staphylococcus aureus-induced pneumonia. Antimicrob Agents Chemother 2014; 58(2):1108-17; PMID:24295977; https://doi.org/10.1128/AAC.02190-13

[11] Flego M, Ascione A, Cianfriglia M, Vella S. Clinical development of monoclonal antibody-based drugs in HIV and HCV diseases. BMC Med 2013; 11:4; PMID:23289632; https://doi.org/10.1186/1741-7015-11-4

[12] Patel G, Bonomo RA. "Stormy waters ahead:" global emergence of carbapenemases. Frontiers Microbiol 2013; 4:48; PMID:23504089; https://doi.org/10.3389/fmicb.2013.00048

[13] Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis. 2013; 13(9):785-96; PMID:23969216; https://doi.org/10.1016/S1473-3099(13)70190-7

[14] Szijarto V, Guachalla LM, Hartl K, Varga C, Banerjee P, Stojkovic K, Kaszowska M, Nagy E, Lukasiewicz J, Nagy G. Both clades of the epidemic KPC-producing Klebsiella pneumoniae clone ST258 share a modified galactan O-antigen type. Int J Med Microbiol: IJMM 2016; 306 (2):89-98; PMID:26723873; https://doi.org/10.1016/j.ijmm.2015.12.002

[15] Abboud N, Chow SK, Saylor C, Janda A, Ravetch JV, Scharff MD, Casadevall A. A requirement for FcgammaR in antibody-mediated bacterial toxin neutralization. J Exp Med 2010; 207(11):2395-405; PMID:20921285; https://doi.org/10.1084/jem.20100995

[16] Weber S, Tian H, van Rooijen N, Pirofski LA. A serotype 3 pneumococcal capsular polysaccharide-specific monoclonal antibody requires FcgammaR receptor III and macrophages to mediate protection against pneumococcal pneumonia in mice. Infect Immun 2012; 80(4):1314-22; PMID:22290146; https://doi.org/10.1128/IAI.06081-11

[17] Tian H, Weber S, Thorkildson P, Kozel TR, Pirofski LA. Efficacy of opsonic and nonopsonic serotype 3 pneumococcal capsular polysaccharide-specific monoclonal antibodies against intranasal challenge with Streptococcus pneumoniae in mice. Infect Immun 2009; 77(4):1502-13; PMID:19168739; https://doi.org/10.1128/IAI.01075-08

[18] Casadevall A, Dadachova E, Pirofski LA. Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2004; 2(9):695-703; PMID:15372080; https://doi.org/10.1038/nrmicro974

[19] Smith P, DiLillo DJ, Bournazos S, Li F, Ravetch JV. Mouse model recapitulating human Fcgamma receptor structural and functional diversity. Proc Natl Acad Sci
[20] Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 2008; 8 (1):34-47; PMID:18064051; https://doi.org/10.1038/nri2206

[21] Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. Blood 2012; 119(24):5640-9; PMID:22535666; https://doi.org/10.1182/blood-2012-01-380121

[22] Doyle CR, Pirofski LA. Reduction of Streptococcus pneumoniae Colonization and Dissemination by a Nonopsonic Capsular Polysaccharide Antibody. MBio 2016; 7(1):e02260-15; PMID:26838726; https://doi.org/10.1128/mBio.02260-15

[23] Roche AM, Richard AL, Rahkola JT, Janoff EN, Weiser JN. Antibody blocks acquisition of bacterial colonization through agglutination. Mucosal Immunol 2015; 8(1):176-85; PMID:24962092; https://doi.org/10.1038/mi.2014.55

[24] Mitsi E, Roche AM, Reine J, Zangari T, Owugha JT, Pennington SH, Gritzfeld JF, Wright AD, Collins AM, van Selm S, et al. Agglutination by anti-capsular polysaccharide antibody is associated with protection against experimental human pneumococcal carriage. Mucosal Immunol 2016; 10(2):385-94; PMID:27579859; https://doi.org/10.1038/mi.2016.71

[25] Diago-Navarro E, Calatayud-Baselga I, Sun D, Khairallah C, Mann I, Ulacia-Hernando A, Sheridan B, Shi M, Fries BC. Antibody-Based Immunotherapy To Treat and Prevent Infection with Hypervirulent Klebsiella pneumoniae. Clin Vaccine Immunol 2017; 24(1); PMID:27795303; https://doi.org/10.1128/CVI.00456-16

[26] Hurley JC. Towards clinical applications of anti-endotoxin antibodies; a re-appraisal of the disconnect. Toxins 2013; 5(12):2589-620; PMID:24351718; https://doi.org/10.3390/toxins5122589

[27] Buchwald UK, Pirofski L. Immune therapy for infectious diseases at the dawn of the 21st century: the past, present and future role of antibody therapy, therapeutic vaccination and biological response modifiers. Curr Pharm Des 2003; 9(12):945-68; PMID:12678861; https://doi.org/10.2174/1381612033455189