IgM Monoclonal Antibodies Targeting Peptidoglycan May Provide Therapeutic Strategies against Antimicrobial Resistant Bacteria

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Background

Antimicrobial resistance (AMR) poses a substantial global threat to human health and development. In addition to death and disability, the cost of AMR to the global economy is significant. Prolonged illness results in longer hospital stays and the need for more expensive medicines and financial challenges for those impacted. Therapeutics such as monoclonal antibodies (mAbs) may offer prevention and control measures against microbial infections without the use of antibiotics. In this study, we developed human antibodies (serum and mAbs) against components of *Staphylococcus aureus* (SA) and *Mycobacterium tuberculosis* (MTB) and evaluated their capabilities.

Methods

- Humanized DRAGA mice were immunized with 20µg of a combination vaccine comprised of Ultrapure peptidoglycan (PGN, derived from SA) and TB Pep01 peptide (targeting MTB HSP16.3), formulated with AddaVax™ adjuvant.
- Serum antibody responses to PGN, TB Pep01, and whole bacteria were analyzed using ELISA.
- Mice with high antisera titers were selected for hybridoma production, and were screened for binding to PGN, TB Pep01, and whole bacteria using ELISA, and high producing clones were selected for monoclonal antibody development.
- Purified mAb was analyzed for recognition of live bacteria including *Mycobacterium smegmatis*, *Staphylococcus epidermidis*, and *Staphylococcus aureus.*
- Opsonophagocytic Killing Activity (OPKA) of purified mAb against live mycobacteria was assessed.

Results

- Humanized DRAGA mice preferentially make IgM antibodies.
- Early and enhanced serum IgM responses to PGN were observed by Day-21 (Figure 1). Serum IgG responses to PGN were detected at Day-35 (Figure 2).
- Antisera binding to TB Pep01 was shown, albeit lower than PGN (Figures 1 & 2).
- Antisera recognition of whole bacteria was shown (Figure 3).

Antisera Binding Activity

Figure 1

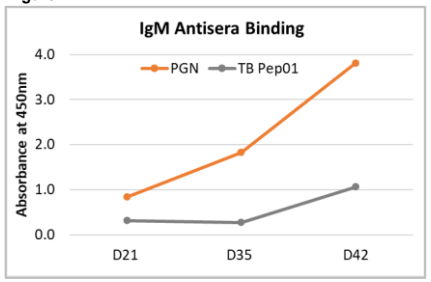


Figure 2

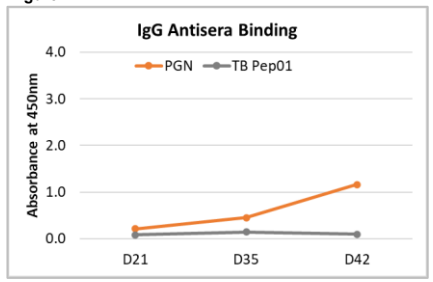
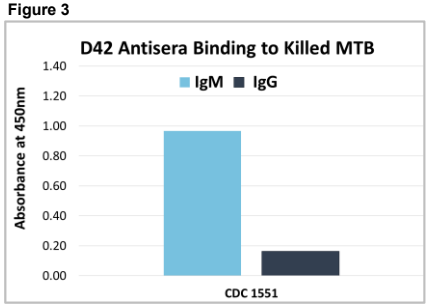


Figure 3



Profile of serum antibody responses to PGN and TB Pep 01 was analyzed using IgM **(Figure 1)** and IgG detection antibodies **(Figure 2)**. Day-42 serum antibody responses to MTB CDC1551 is shown **(Figure 3)**.

mAb Binding Activity

Figure 4

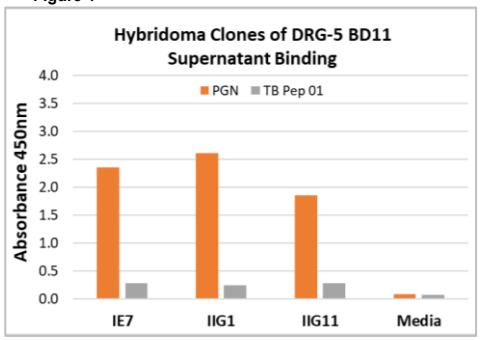
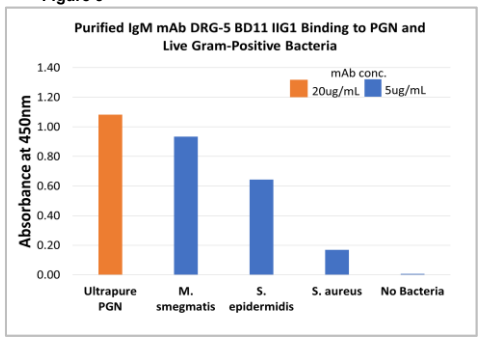


Figure 5



Hybridoma Clones DRG-5 BD11 supernatant binding activity to PGN and TB Pep 01 **(Figure 4).** Human IgM mAb DRG-5 BD11 IIG1 binding activity to PGN and various live gram-positive bacteria at 10^5 CFU/mL **(Figure 5)**.

mAb Functional Activity

Table 1

mAb ug/mL	Peak OPKA
31	58%
2	50%
1	44%
0.5	49%

Preliminary functional activity of human IgM mAb DRG-5 BD11 IIG1 against mycobacteria (*M. smegmatis*) showed significant[1] OPKA at 2 and 31 ug/mL using U-937 macrophages.

Results cont.

- Hybridoma DRG-5 BD11 clones (IgM) targeting PGN were identified for monoclonal antibody production (Figure 4).
- Purified IgM mAb DRG-5 BD11 IIG1 bound to ultrapure PGN, and to live gram-positive bacteria (Figure 5).
- Purified IgM mAb DRG-BD11 IIG1 significantly[1] enhanced killing of mycobacteria using U-937 macrophages (Table 1).

Conclusions

- IgM mAb DRG-5 BD11 IIG1 developed from humanized DRAGA mice immunized with PGN and TBPep01 showed broad recognition of various microbes and enhanced killing of mycobacteria. Ongoing studies to evaluate mAb functional activity against other microbes are in progress.
- IgM mAbs that recognize whole bacteria, and opsonize and kill multiple bacterial strains, may provide an effective antimicrobial strategy for treatment of drug resistant bacterial infections.[2]

References

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[2]Keyt, Bruce A., et al. "Structure, function, and therapeutic use of IgM antibodies." *Antibodies* 9.4 (2020): 53.