Opsonic Monoclonal Antibodies Directed Against MTB Enhance Blood Clearance in a Quantitative qPCR Mouse Model

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BACKGROUND

In sub-Saharan Africa, *Mycobacterium tuberculosis* (MTB) is an important cause of bacteremia and sepsis. Patients with HIV and MTB bacteremia have a high mortality with many deaths occurring within 18 days of presentation[1]. Therefore, rapid diagnosis of MTB bacteremia is critical and can be achieved using real-time quantitative PCR (qPCR). Treatment of MTB sepsis with immunotherapeutic agents may enhance clearance of MTB bacteremia and augment therapy for MDR and XDR TB.

METHODS

Monoclonal Antibody (MAB) Identity and Characterization: Hybridoma supernatant and purified MABs GG9, AB9 and JG7 were screened by ELISA for their binding activity to several MTB strains (Erdman, HN878, CDC1551) and *M. smegmatis*. The latter non-pathogenic strain was used as a surrogate *Mycobacteria* strain in the opsonophagocytic assay.

Opsonophagocytic Killing Activity (OPKA): The functional activity of MABs GG9, AB9 and JG7 (@25–0.06ug/mL) was evaluated using Human Leukemia Promyelocytic cell line (HL60) and Human Histiocytic Lymphoma cell line (U-937), against *Mycobacterium smegmatis*. MABs were reacted with differentiated effector cells in the presence (for HL60s) or absence (for U-937s) of complement component C1q and *M. smegmatis*. OPKA was defined as the percentage of the average CFU counts in test sample wells (with MAB) divided by average control CFU counts (without MAB). When bacterial CFU was reduced by greater than 50%, OPKA was considered antibody enhanced[2].

MTB Challenge/MAB Treatment: Female ICR mice were given opsonic anti-MTB MAB GG9 intraperitoneally at dose levels 10mg/kg, 5mg/kg, 1mg/kg, and placebo (PBS) twenty-four hours prior to MTB HN878 challenge. Challenge doses were at 10^5 and 10^8 CFU/mL, administered intravenously. Whole blood specimens were collected at 0, 4, and 24 hours post-challenge, placed in K2-EDTA and then 0.1 mL added into 1 mL PrimeStore MTM® prior to shipping at ambient temperature from Gaithersburg, Maryland to San Antonio, Texas for quantitative qPCR testing.

DNA Extraction/qPCR: Total nucleic acid (DNA) was extracted from 0.2mL murine blood specimen using PrimeXtract™. Real-time qPCR was performed by adding 2.5 µL of specimen into 7.5uL of PrimeMix® MTB Multiplex (IS6110/1S1081) on an ABI 7500.

MTB Blood Clearance: The level of MTB in murine blood was monitored by qPCR and total clearance was defined as cycle threshold (C_T) = 40 for both IS-6110 and IS-1081 targets. Group average C_T values, standard deviation and percentage of mice with total clearance was recorded.

RESULTS

MABs GG9, AB9 and JG7 bind to killed *Mycobacterial tuberculosis* strains (Erdman, HN878, and CDC1551; Figure 1) and live *M. smegmatis* (Figure 2) as screened using ELISA.

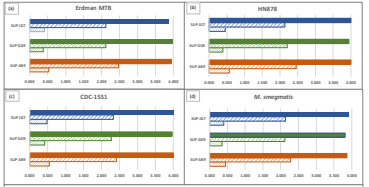

Figure 1. Binding activity profile of Supernatant from JG7, GG9, and AB9 Hybridoma cells to *M. tuberculosis* and *M. smegmatis*. Supernatants were tested by ELISA at dilutions 1:10, 1:100 and 1:1000 on fixed mycobacteria at 1x10^7 CFU/well. Panel (a) through (c), respectively, shows binding of supernatant to inactivated MTB strains Erdman: EtOH-Killed, HN878: Gamma Irradiated, and CDC-1551: Gamma Irradiated. Panel (d) depicts binding to fixed *M. smegmatis*. Supernatant from JG7, GG9 and A89 hybridoma cells bound to *M. tuberculosis* and *M. smegmatis*.

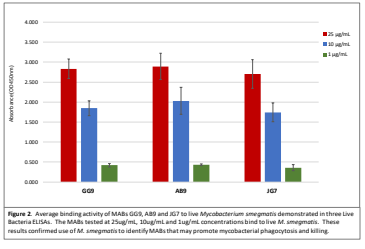

Figure 2. Average binding activity of MABs GG9, AB9 and JG7 to live *Mycobacterium smegmatis* demonstrated in three Live Bacteria ELISAs. The MABs tested at 25ug/mL, 10ug/mL and 1ug/mL concentrations bind to live *M. smegmatis*. These results confirmed use of *M. smegmatis* to identify MABs that may promote mycobacterial phagocytosis and killing.

MABs GG9, AB9 and JG7 have shown OPKA > 50% (across high and low concentrations) using both granulocytes and macrophages. Peak OPKA for GG9 was 67% (1ug/mL); AB9, 75% (25ug/mL); and JG7, 81% (0.06ug/mL) using differentiated HL60 cells (Figure 3). With U-937s, MABs GG9 and AB9 had peak OPKA of 55% at 1.5ug/mL (data not shown).

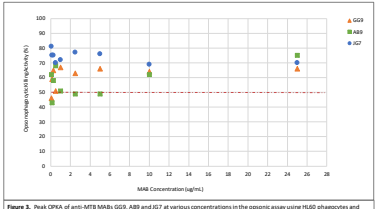

Figure 3. Peak OPKA of anti-MTB MABs GG9, AB9 and JG7 at various concentrations in the opsonic assay using HL60 phagocytes and C1q, against *Mycobacterium smegmatis*. Complement component C1q did not mediate mycobacterial killing in the absence of antibody. All three MABs enhanced mycobacterial phagocytosis and had maximum killing of 67% for GG9, 75% for AB9, and 81% for JG7.

Female ICR mice given MAB GG9 at doses 10mg/kg and 5mg/kg had enhanced blood clearance of killed HN878 bacilli by twenty-four hours post-challenge with both a low (10^5 CFU/mL) and high (10^8 CFU/mL) MTB dose compared to the placebo (PBS) groups (Figure 4). Treatment with GG9 at 5mg/kg showed total clearance (C_T = 40 for IS6110) in 7/9 mice.

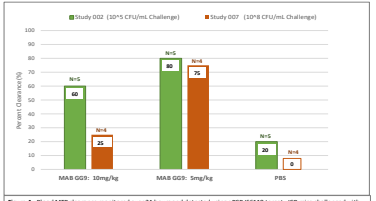

Figure 4. Blood MTB clearance monitored over 24 hours and detected using qPCR IS6110 target. ICR mice challenged with 10^5 and 10^8 CFU/mL gamma-irradiated HN878 bacilli and treated with anti-MTB MAB GG9 had 60% (3/5 mice); 25% (1/4 mice) - given 10mg/kg and 80% (4/5 mice); 75% (3/4 mice) - given 5mg/kg clear blood MTB by 24 hours (C_T=40 for IS6110), respectively. In comparison, the placebo groups (PBS) challenged with 10^5 and 10^8 CFU/mL had 20% (1/5 mice) and 0% (0/4 mice) clear blood MTB by 24 hours, respectively. Anti-MTB MABs enhanced clearance of MTB in murine blood.

At twenty-four hours post MTB challenge, a steady decline in HN878 bacilli with decreased C_T values was noted in MAB treated mice compared to PBS control (Figure 5). Duplicate averages with standard error are indicated.

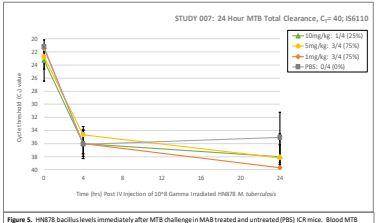

Figure 5. HN878 bacillus levels immediately after MTB challenge in MAB treated and untreated (PBS) ICR mice. Blood MTB clearance monitored over 24 hours and detected using qPCR IS6110 target. Mice treated with anti-MTB MAB GG9 at 10mg/kg, 5mg/kg and 1mg/kg had a steady decline in HN878 bacilli between 4 and 24 hours compared to the placebo group (PBS).

CONCLUSIONS

- ✓ Anti-MTB MABs GG9, AB9 and JG7 were identified that bind to MTB strains and promote mycobacterial OPKA.
- ✓ Using qPCR to detect and monitor killed MTB in murine blood is an efficient methodology to identify MABs that enhance clearance of MTB.
- ✓ Anti-MTB MABs, like GG9, may enhance clearance of MTB bacteremia and might provide useful adjunctive therapy for MTB sepsis.
- ✓ Anti-MTB MABs may also be useful for therapy in patients with MDR and XDR TB.
- ✓ Current studies are being conducted using live MTB strains to further identify potential therapeutic MAB candidates.

REFERENCES

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[2] Fleck RA, Romero-Steiner S and Nahm MH. (2005) Use of HL-60 Cell Line to Measure Opsonic Capacity of Pneumococcal Antibodies. CLIN DIAGN LAB IMMUN, p. 19-27.

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