Chitin Derived Small Molecule AVR-48 Reprograms the Resting Macrophages to an Intermediate Phenotype and Decrease *Pseudomonas aeruginosa* Mouse Lung Infection

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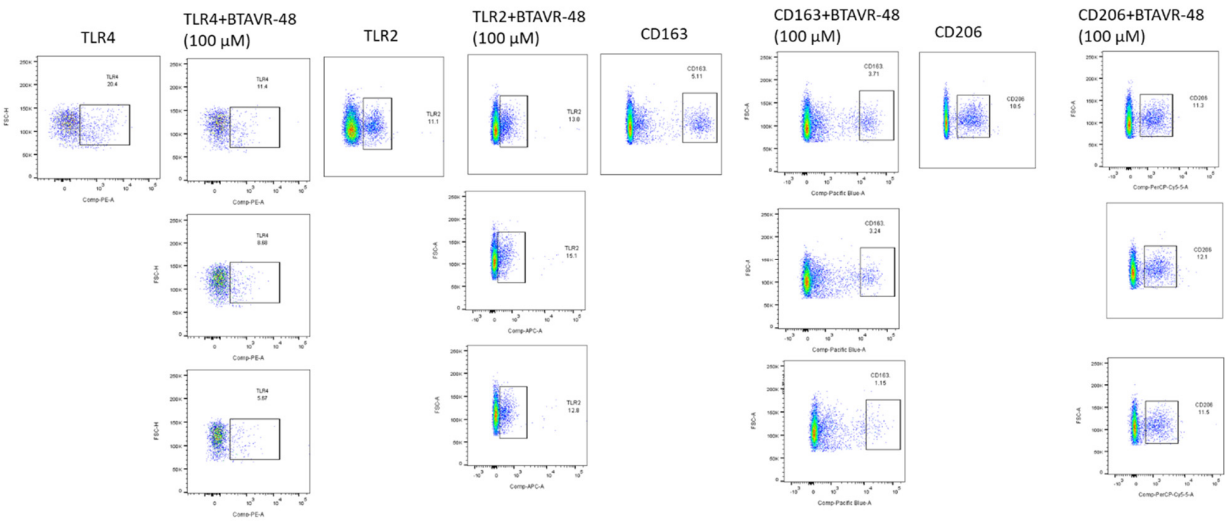
Supplemental Figures

1.1. AVR-48 selectively bind to TLR4 and CD163 and not to TLR2 or CD206 in mouse spleen derived mononuclear cells

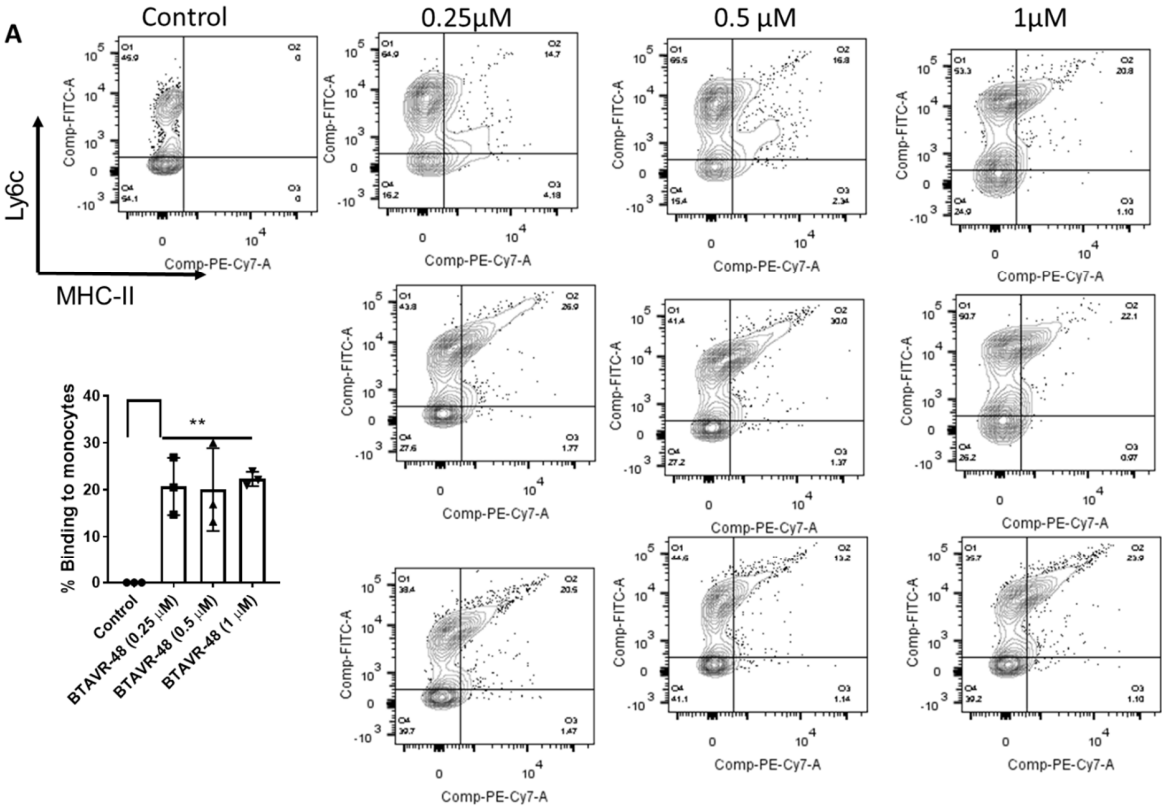


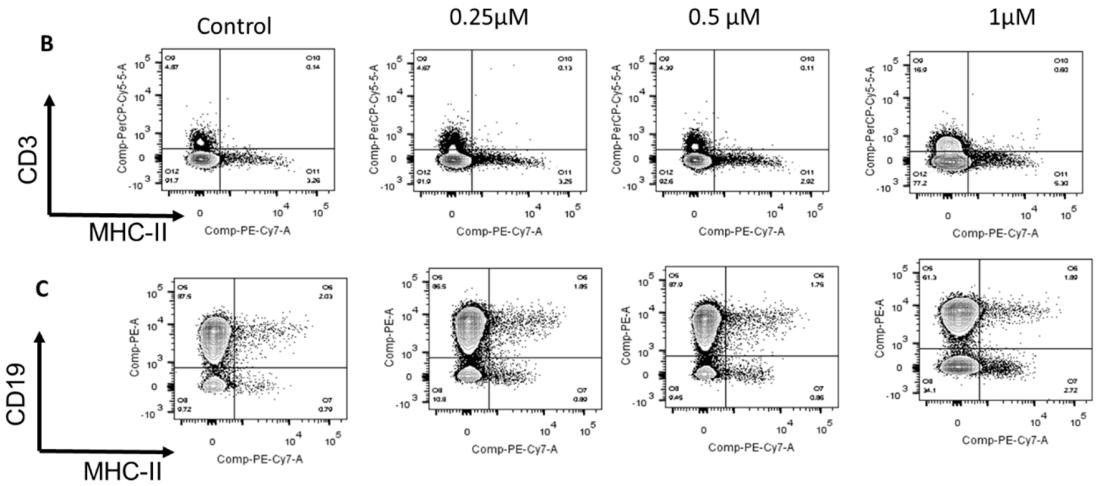
Supplemental Figure S1: Binding study of biotin conjugated AVR-48 to mouse monocytes.

Primary splenocytes of C57BL/6J mice (n=3) were incubated at 4ºC for 1 hr followed by

incubation with biotinylated AVR-48 (BTAVR-48) at different concentrations along with monocyte (Ly6C) marker. The cells were probed with appropriate fluorescence coupled streptavidin (for anti-mouse TLR4, TLR2, CD163 and CD206 antibodies) and percentage of cells is analyzed by FACS. Data analyzed by FlowJo Software. The data presented is representative of one out of the two experiments

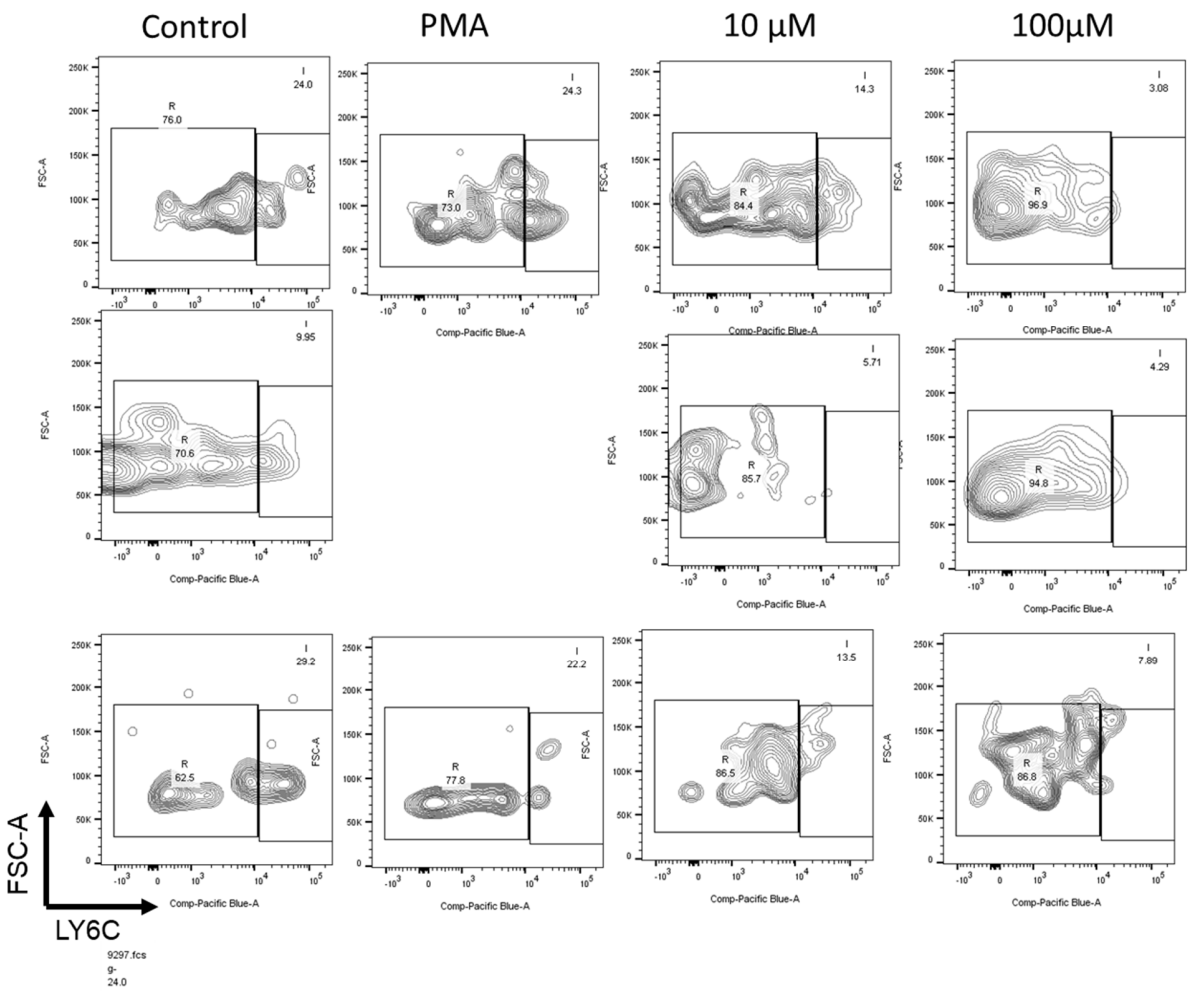
1.2. AVR-48 has binding affinity for monocytes but not to T cells or B cells derived from mouse splenocytes





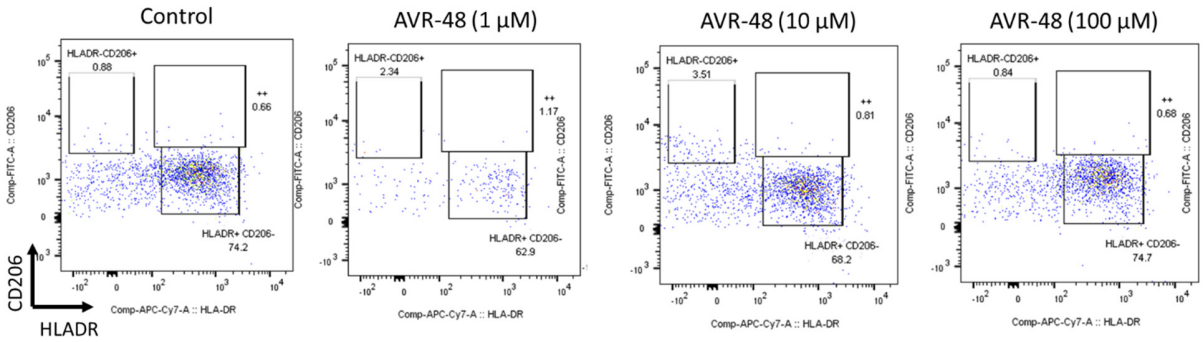
Supplemental Figure S2: Binding of biotinylated conjugated AVR-48 to splenic monocytes, T cells and B cells. Briefly, cells were incubated at 4ºC for 1 hr followed by incubation with biotinylated AVR-48 (0.25 µM, 0.5 µM, 1.0 µM) along with monocyte (Ly6C) markers (A), T cell (CD3) marker (B) and B cell (CD19) marker (C). Then the cells were probed with appropriate fluorescence coupled streptavidin and analyzed by FACS. Dead cells were excluded during analysis. N =3. The data presented is representative of one out of the two experiments.

1.3. AVR-48 treatment increased resident monocytes in mouse spleen cells after 72h (Raw FACS data)



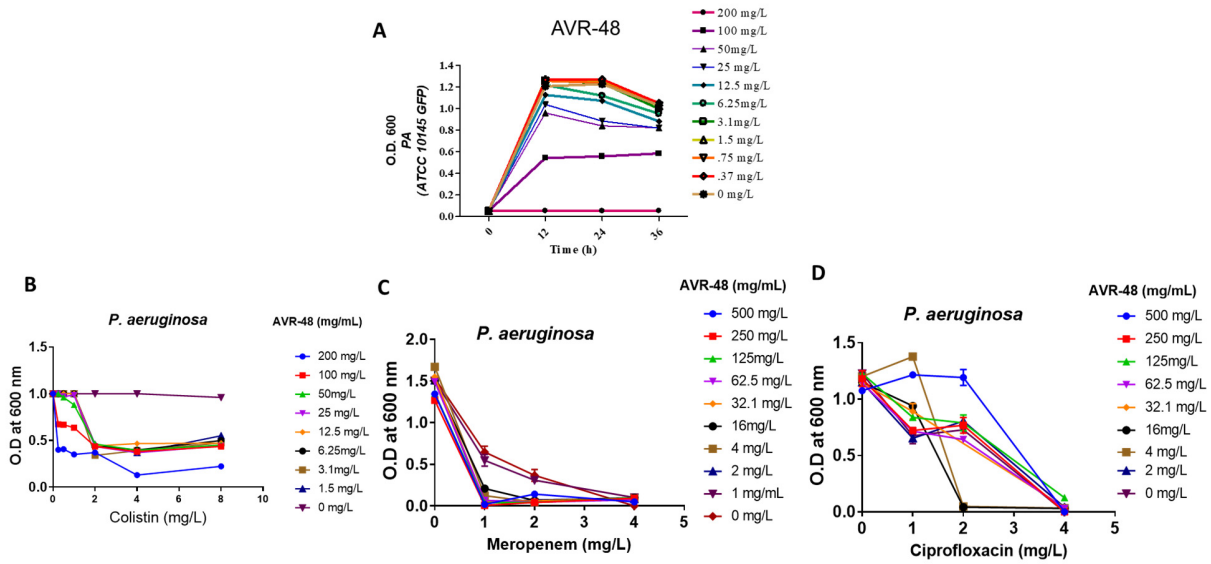
Supplemental Figure S3: AVR-48 treatment increased resident monocytes in mouse spleen cells after 72h (Raw FACS data for Figure 5). The data presented is representative of one out of the two experiments.

1.4. Treatment of AVR-48 to human peripheral blood mononuclear cells (hPBMCs) for 48h



Supplemental Figure S4: Flow cytometry data after treatment of AVR-48 to hPBMCs for 48h. Treatment of AVR-48 (1, 10, 100 µM) to hPBMCs for 48h followed by staining and FACS didn't increase the % of intermediate macrophages (HLA-DR^{+} CD206^{+}). The major % of macrophages were found to be M1 macrophages (HLA-DR^{+} CD206^{-}). N =2 technical replicates and the experiment is repeated three times. The data presented is representative of one out of the three experiments

1.5. Combination MIC and synergy of AVR-48 and different standard of care antibiotics



Supplemental Figure S5: Determination combination minimum inhibitory concentrations (MIC_{90}) of AVR-48 and different antibiotics for *Pseudomonas aeruginosa* using broth dilution assay. A) MIC of AVR-48 for *P.aeruginosa* was determined to be 200 µg/mL. B) In absence of AVR-48, the MIC of colistin is 8 mg/mL, when combined with 3.1 mg/mL of AVR-48, the MIC of colistin is decreased 4-fold (2 mg/mL). C) In absence of AVR-48, the MIC of meropenem is 4 mg/mL; when combined with 4.0 mg/mL of AVR-48, the MIC of meropenem is decreased 4-fold (1 mg/mL). C) In absence of AVR-48, the MIC of ciprofloxacin is 4 mg/mL, when combined with 4.0 mg/mL of AVR-48, the MIC of ciprofloxacin is decreased 2-fold (2mg/mL). N =4 technical replicates. The experiment is repeated 3 times. The data presented is representative of one out of the three experiments