Synergy between immune system and antibiotics drives infection control in mice

Rajalekshmy G. Padmakumari [1] , Ruchi Roy [2] , Foyez Mahmud [3] , Deepa Dehari [1] , Getnet Tesfaw Tadege[1‡] , Christi Thomas [1] , Athena M. Soulika [1,4], Roslyn Rivkah Isseroff [1,5] , Sasha H. Shafikhani [1,6,7,8*]

Affiliations

1 Department of Dermatology, University of California Davis, Sacramento, CA, United States, 2 UICentre for Drug Discovery, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, United States, 3 VeriSim Life Inc., San Francisco, CA, United States, 4 Shriners Hospital for Children, Sacramento, CA, United States, 5 Dermatology Section, VA Northern California Health Care System, Mather, CA, United States, 6 Microbiology Graduate Group (MGG), University of California Davis, Sacramento, CA, United States, 7 Graduate Group in Immunology (GGI), University of California Davis, Sacramento, CA, United States, 8 Cancer Center, University of California Davis, Sacramento, CA, United States

‡ Presenting author, * Corresponding author: sshafikhani@ucdavis.edu

INTRODUCTION

- **Observation**: Systemic antibiotics often fail to control infections even if the causative pathogens are sensitive to the antibiotics.
- **Current paradigm**: Antibiotics and immune system are assumed to function independently in controlling infection. The idea is that antibiotics lower bacterial burden to a point that can be managed by immune system.
- **Hypothesis**: We believe that there is a direct cross-talk between antibiotics and innate immune system that is essential for controlling infection and this synergy is broken in immunocompromised patients.

PROJECT HYPOTHESIS

- We hypothesize that the initial bacterial killing by antibiotics results in increased bioactive (bioavailable) PAMPs that trigger a self-sustaining innate immune response at the site of infection, which combat infection.
- This synergy link is broken in immunocompromised patients.
- We also posit that live pathogens do not activate PRR-mediated signaling as much as dead (lysed) bacteria because their ligands are not free to interact with PRRs.

EXPERIMENTAL DESIGN

Day (0):

1) Mice (normal and immunocompromised) received antibiotics (Tob) or PBS control
2) 1 hour after antibiotics, mice were wounded (5-mm punch)
3) Wounds were treated with: PA103 (10^6/wound)

Assessments & Endpoints (Day 0 & Day 1)

Wound tissues harvested and assessed for:

1) Infection titer (CFU counts)
2) Proinflammatory markers (TNFα and) IL1β by ELISA) and Neutrophil activation marker (MPO by ELISA)
3) Inflammatory responses (H&E and IHC)
4) LPS bioactive level measurements by HEK-Blue reporter cells

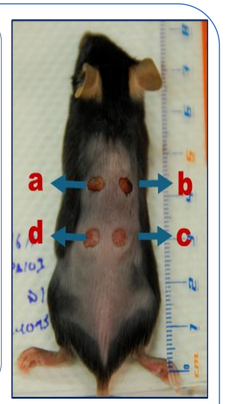

Fig. 2: Excisional full-thickness mouse wound models. Four equidistant wounds were created on the dorsal side of the mouse with a surgical biopsy punch (5 mm diameter).

RESULTS & DISCUSSIONS

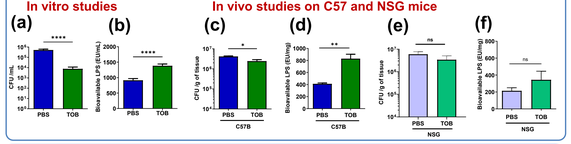

Fig. 3: ***P. aeruginosa* bacterial killing by Tobramycin increases bioactive (bioavailable) LPS levels in C57BL wounds, but not in NSG wounds.** *(a-b)* PA103 bacteria cultured in LB (10^8 bacteria/mL) were treated with Tobramycin (3.5 μg/mL) or PBS for 1 h. Viable bacteria were quantified by CFU determination *(a)*, and bioactive LPS was measured using HEK-Blue hTLR4 reporter cells *(b) (N=6 replicates)*. *(c-d)* C57BL/6 and NSG mice received PBS or Tobramycin (3.5 mg/kg) by i.p. injection 1 h prior to wounding and infection with PA103 (10^6 bacteria/wound). Wound tissues collected after 1 h were analyzed for bacterial burden by CFU assay, normalized to tissue weight *(c, e)*, and for bioactive LPS using HEK-Blue reporter cells *(d, f)*. Data were normalized to wound tissue weight (N=4 mice/group). Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test (ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

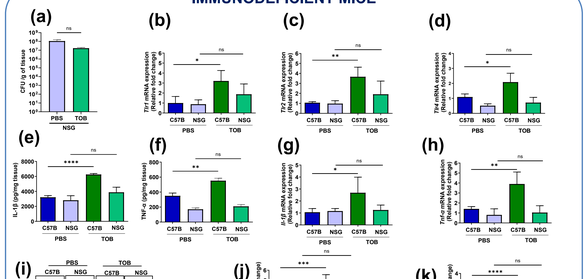

Fig. 5: **Tobramycin treatment fails to boost immunity against *P. aeruginosa* infection in NSG wounds.** Mice were injected intraperitoneally with tobramycin (TOB) (3.5 mg/kg) or PBS (mock) 1h prior to wounding. Wounds from C57B and NSG mice were infected with PA103 (10^6 CFU/wound). The wounds were collected after 24h and assessed for: *(a)* bacterial content by CFU count; *(b-d)* mRNA expression of Toll-like receptors (TLRs): *Tlr1*, *Tlr2* and *Tlr4* by RT-PCR (normalized to 18S); *(e-h)*: proinflammatory cytokines IL-1β and TNF-α levels by ELISA *(e-f)* and by m-RNA transcription analysis by RT-PCR *(g-h)*; Western blot analysis of proinflammatory cytokines and the corresponding densitometer tabulated data for cytokines *(i-k)* IL-1β and k) TNF-α are shown as the relative fold change. Leukocytes contents assessed by histological analysis using H&E staining *(l, m)*, activated neutrophils contents by immunohistochemistry using anti-Ly6G antibody *(n, o)* and MPO ELISA *(p)*. Red scale bars = 50μm. Corresponding data were plotted as the Mean ± SEM. (N=4). Statistical comparison between groups was determined using one-way ANOVA with Tukey's post hoc test (ns= not significant, *p<0.05, **p<0.01, ***p<0.001

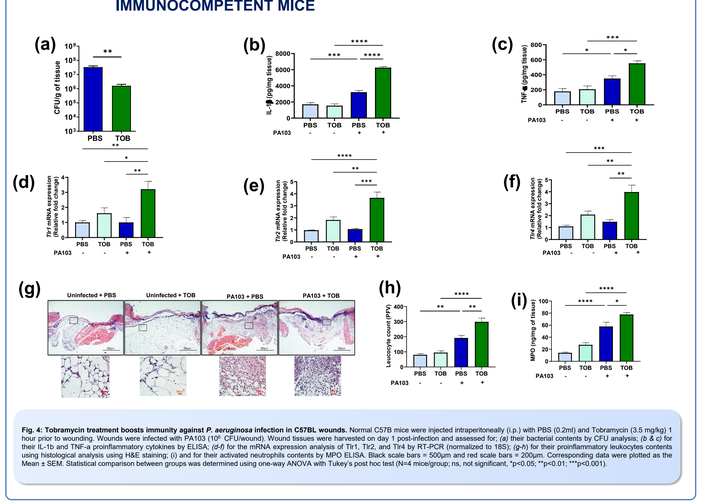

Fig. 4: Tobramycin treatment boosts immunity against *P. aeruginosa* infection in C57BL wounds. Normal C57B mice were injected intraperitoneally (i.p.) with PBS (0.2ml) and Tobramycin (3.5 mg/kg) 1 hour prior to wounding. Wounds were infected with PA103 (10^6 CFU/wound). Wound tissues were harvested on day 1 post-infection and assessed for: *(a)* their bacterial contents by CFU analysis; *(b & c)* for their IL-1b and TNF-α proinflammatory cytokines by ELISA; *(d-f)* for the mRNA expression analysis of Tlr1, Tlr2, and Tlr4 by RT-PCR (normalized to 18S); *(g-h)* for their proinflammatory leukocytes contents using histological analysis using H&E staining; (i) and for their activated neutrophils contents by MPO ELISA. Black scale bars = 500μm and red scale bars = 200μm. Corresponding data were plotted as the Mean ± SEM. Statistical comparison between groups was determined using one-way ANOVA with Tukey's post hoc test (N=4 mice/group; ns, not significant, *p<0.05; **p<0.01; ***p<0.001).

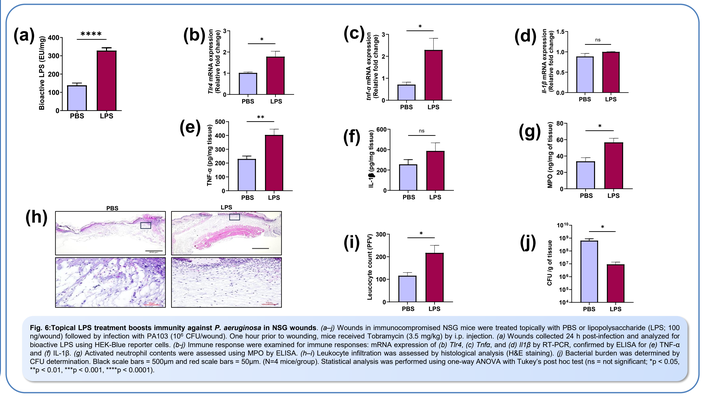

Fig. 6:**Topical LPS treatment boosts immunity against *P. aeruginosa* in NSG wounds**. *(a-j)* Wounds in immunocompromised NSG mice were treated topically with PBS or lipopolysaccharide (LPS; 100 ng/wound) followed by infection with PA103 (10^6 CFU/wound). One hour prior to wounding, mice received Tobramycin (3.5 mg/kg) by i.p. injection. *(a)* Wounds collected 24 h post-infection and analyzed for bioactive LPS using HEK-Blue reporter cells. *(b-j)* Immune response were examined for immune responses: mRNA expression of *(b) Th1*, *(c) Th5c*, and *(d) Il1β* by RT-PCR, confirmed by ELISA for *(e)* TNF-α and *(f)* IL-1β. *(g)* Activated neutrophil contents were assessed using MPO by ELISA. *(h–j)* Leukocyte infiltration was assessed by histological analysis (H&E staining). *(j)* Bacterial burden was determined by CFU determination. Black scale bars = 500μm and red scale bars = 50μm. (N=4 mice/group). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test (ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

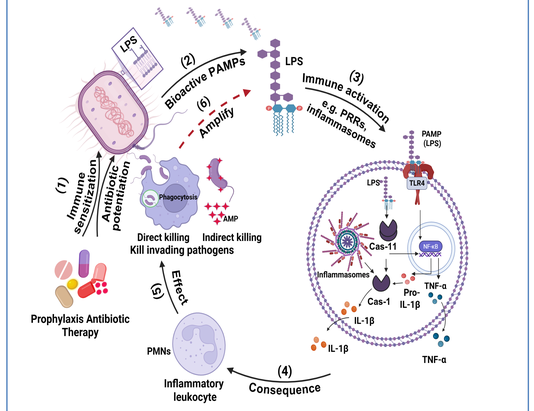

Fig.1. Schematic diagram of the model underlying the synergistic interaction between antibiotics and the immune system. In this model, antibiotic-induced bacterial lysis is likely enhanced by the immune system sensitization, leading to the release of bioactive pathogen-associated molecular patterns (PAMPs) (1; Immune sensitization/Antibiotic potentiation). This results in elevated levels of bioavailable/bioactive PAMPs at the infection site (2, Bioactive PAMPs), which subsequently activate immune pathways, such as pattern recognition receptors (PRRs) and inflammasomes (3). Activation of these pathways increases the production of proinflammatory cytokines such as TNF-α and IL-1β at the infection site (3, PRRs & Inflammasomes activation). These cytokines facilitate the recruitment of inflammatory leukocytes, especially neutrophils, to the infected area (4; Consequence). The recruited neutrophils directly kill bacteria (5; Effect) while also amplifying inflammation through further cytokine release (6; Amplification). Together, this immune-mediated synergy enhances the in vivo effectiveness of antibiotics.

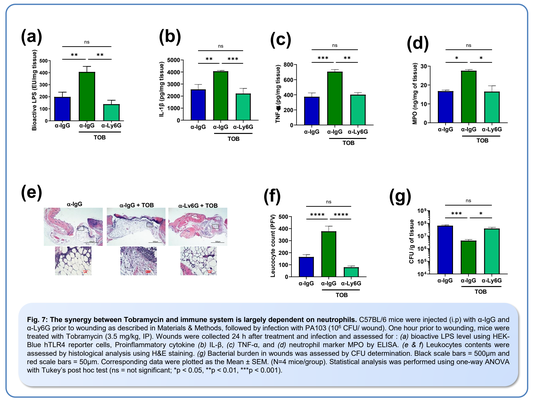

Fig. 7: The synergy between Tobramycin and immune system is largely dependent on neutrophils. C57BL/6 mice were injected (i.p) with α-IgG and α-Ly6G prior to wounding as described in Materials & Methods, followed by infection with PA103 (10^6 CFU/ wound). One hour prior to wounding, mice were treated with Tobramycin (3.5 mg/kg, IP). Wounds were collected 24 h after treatment and infection and assessed for : *(a)* bioactive LPS level using HEK-Blue hTLR4 reporter cells. Proinflammatory cytokine *(b)* IL-β, *(c)* TNF-α, and *(d)* neutrophil marker MPO by ELISA. *(e & f)* Leukocytes contents were assessed by histological analysis using H&E staining. *(g)* Bacterial burden in wounds was assessed by CFU determination. Black scale bars = 500μm and red scale bars = 50μm. Corresponding data were plotted as the Mean ± SEM. (N=4 mice/group). Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test (ns = not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

CONCLUSION

- In C57BL/6 mice, tobramycin-mediated bacterial killing increased pathogen-associated molecular patterns (PAMPs) - namely lipopolysaccharide (LPS) - which in turn amplified local inflammation, enhancing antibiotic efficacy.
- The immune neutrophils play a significant role in enhancing the effectiveness of antibiotics in controlling infection.
- However, NSG mice failed to potentiate tobramycin bacterial killing to increase PAMPs and mount a tobramycin-induced boost in immune activation, resulting in reduced infection control.
- Importantly, topical PAMPs (such as LPS) restored immune activation and improved infection control in NSG mice.
- These findings provide direct experimental evidence that antibiotic efficacy requires synergy with host immunity and therapeutic potential of augmenting innate immune activation to improve infection outcomes, particularly in immunocompromised patients.

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