Immunomodulatory Effects of Novel Antimicrobial Peptides Targeting Priority Avian Pathogens

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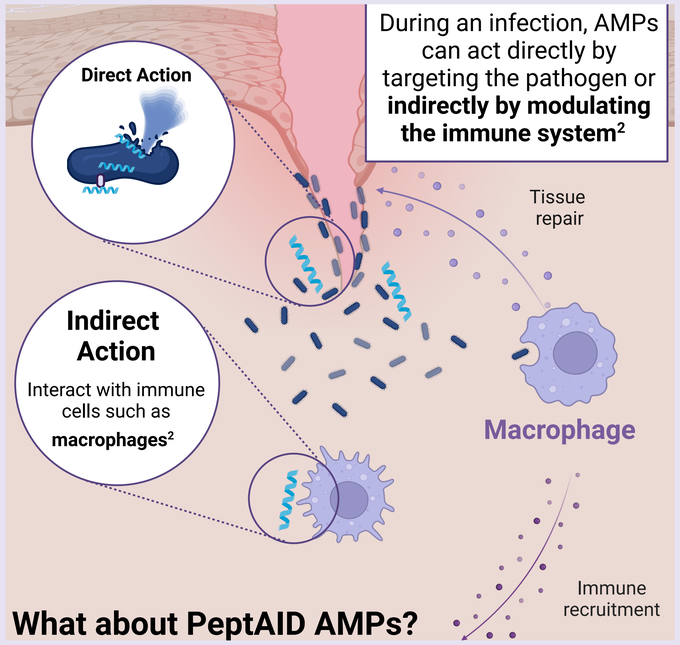
Antimicrobial Resistance Threatens Poultry

Antimicrobial Resistance (AMR) is an escalating global health crisis propelled by the misuse of antibiotics in healthcare and agriculture[1]. Traditional antibiotics typically work through a singular mechanism of action which exerts selective pressure on microbes, favoring those with resistance to antimicrobials[1].

AMPs are Promising Antibiotic Alternatives

Antimicrobial peptides (AMPs) are a promising alternative to traditional antibiotics since they act through multiple mechanisms of action (Figure 1, below)[2]. **PeptAID** is a collaborative project with a rapid pipeline for novel AMP discovery using attentive deep learning models. Our goal is to develop AMPs as antibiotic alternatives in the poultry industry[3].



Do PeptAID AMPs interact with macrophages?

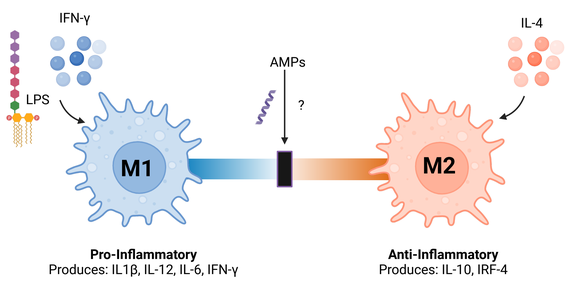


Figure 2. To study the immunomodulatory effects of AMPs, we will examine the impact of AMP treatment on a **chicken macrophage-like cell line (HD11)**. (Above) macrophage polarization along a gradient of M1 and M2 phenotypes[4,5].

Materials and Methods

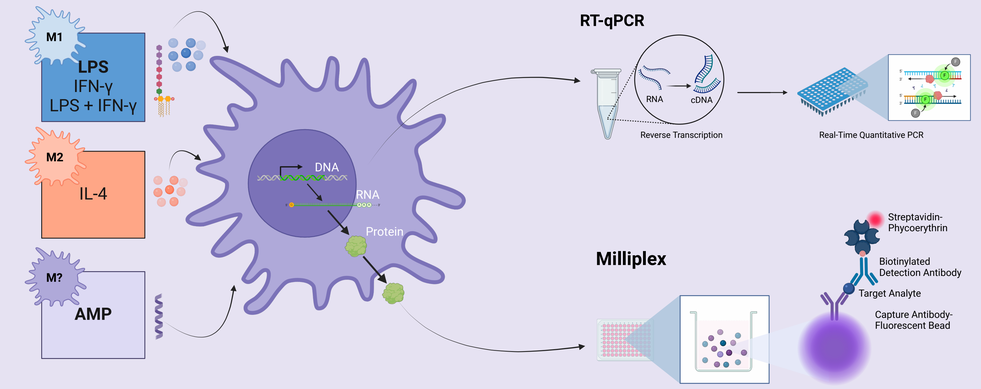


Figure 3. Methods. HD11s will be stimulated and their RNA and secreted proteins will be harvested for analysis by RT-qPCR and Milliplex assays. The Milliplex assay is conducted by Eve Technologies[6].

PeptAID AMPs Activate Chicken Macrophages

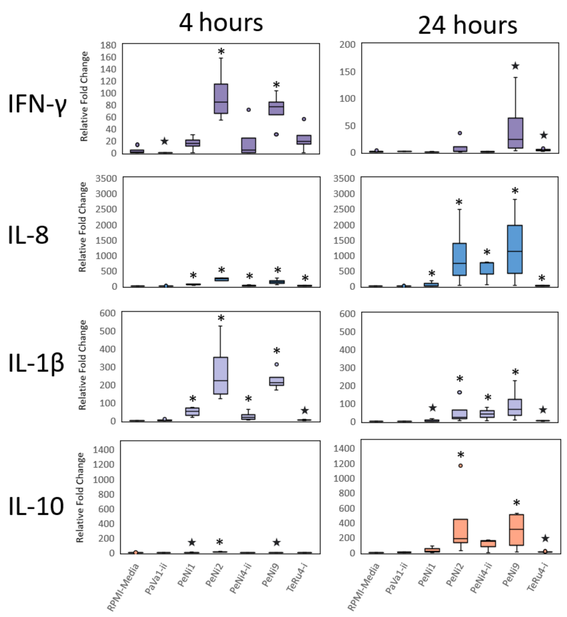


Figure 4. Relative fold change of cytokine transcripts as determined by RT-qPCR after treatment of HD11s with 50 ng/mL LPS, RPMI Media or 32 ug/mL AMP (n=1). LPS-treated samples are not shown, but have relative fold changes of 45 ±8 to 967 ±137 at 4 hours incubation and 19 ±14 to 3794 ±374 at 24 hours incubation. Significant difference from media, * $p \leq 0.05$, ★ $p \leq 0.1$.

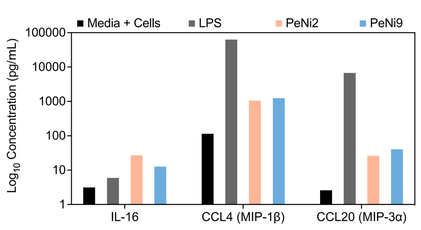


Figure 5. Secreted pro-inflammatory cytokine, IL-16, and chemokines, CCL4 (MIP-1β) and CCL20 (MIP3α) detected using a Milliplex assay from media supernatant after treatment of HD11s with RPMI media, 50 ng/mL LPS, PeNi2 (4 ug/mL) or PeNi9 (2 ug/mL) for 24 hours (n=1).

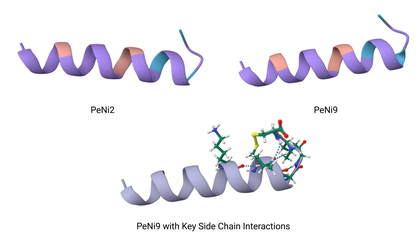


Figure 6. Colabfold structural predictions for PeNi2 and PeNi9. Top figure colours denote basic (orange), polar (blue) and nonpolar (purple) amino acids. Bottom figure demonstrates an internal disulfide bond predicted for both structures. See source 7 for Colabfold details[7].

PeptAID AMPs Attenuate LPS-induced Inflammation

Lipopolysaccharide (LPS) from *Salmonella enterica* and *E. coli* induce a hyperinflammatory response that can be detrimental to the host[10]. PeptAID AMPs attenuate LPS-induced inflammation by down regulating pro-inflammatory cytokines produced by LPS-treated chicken macrophages.

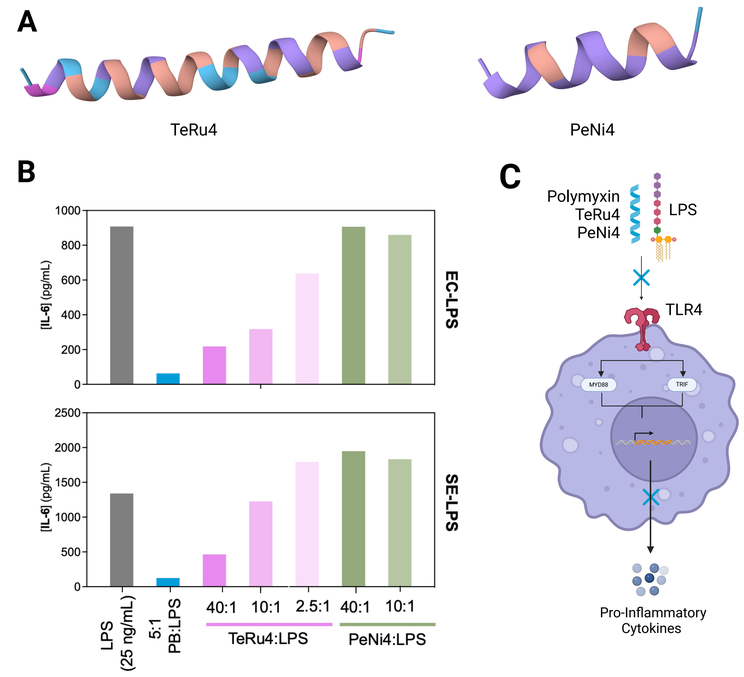


Figure 7. Panel A: Colabfold structural predictions of TeRu4 and PeNi4[2,7] Panel B: TeRu4 may attenuate *E. coli* and *Salmonella enterica* Enteriditis LPS-induced IL-6; polymyxin B (PB) is a last resort antibiotic known to sequester LPS and is shown as a positive control for attenuation of LPS-induced inflammation[11] (n=1). Panel C: Schematic of attenuation of LPS-induced inflammation involving a dampening of TLR4 signaling[4,5,11].

Conclusions and Future Directions

- PeptAID AMPs are capable of stimulating a chicken macrophage-like cell line (HD11)
- TeRu4 may attenuate *Salmonella* and *E. coli* LPS-induced inflammation[10]
- Future experiments will examine the effect of AMPs on macrophages pre-stimulated with IFN-γ, LPS, or IL-4
- Cell-line repertoire will be expanded to include multiple chicken macrophage cell lines and other cell types known to interact with AMPs
- Research findings will help in selecting safe and effective AMPs for *in vivo* trials

Acknowledgements and References

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