FMS-LIKE TYROSINE KINASE-3 LIGAND (FLT3L) REDUCES SYSTEMIC INFECTION IN A MODEL OF POST-BURN PNEUMONIA

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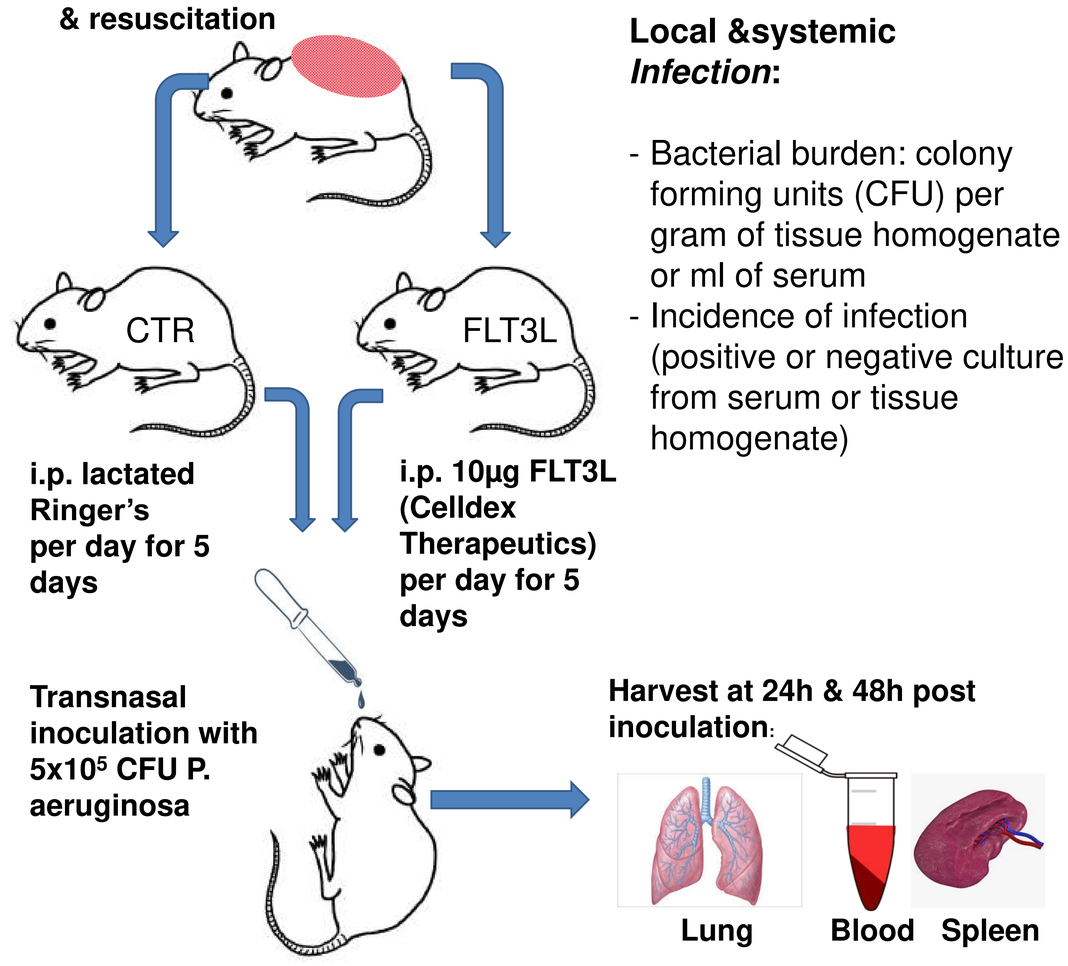
Background

- **Pneumonia** is a common complication of severe burn injury
  - ⇒Incidence: 10-30%[1]
  - ⇒Increased Morbidity & Length of Stay
  - ⇒Mortality Increase: 40-60%[2]
  - ⇒Common Pathogen: *P. aeruginosa*
  - ⇒Increasing emergence of multidrug-resistant strains
- **Immune dysfunction** occurs after burn injury
  - ⇒Hypermetabolism, Hypercatabolism[3]
  - ⇒Surge of proinflammatory cytokines (i.e. IL-6, IL-8) associated with ↑ susceptibility to infection and sepsis[3]
  - ⇒Depletion of dendritic cells, associated with ↑ susceptibility to infection and sepsis[4]
- **FLT3L** (fms-like tyrosine kinase-3 ligand)
  - ⇒promotes expansion of myeloid and lymphoid progenitors
  - ⇒Expands dendritic & NK cell populations[5]
  - ⇒↑ bacterial clearance, ↓ systemic dissemination and ↑ survival in murine post-burn wound infection after prophylactic treatment[5]
  - ⇒Reported increase in mortality and inflammation in pneumonia by Winter et al[6]
- **Hypothesis**

  Prophylactic Treatment with FLT3L Enhances the Immune Response to Post-Burn P. aeruginosa Pneumonia

Methods



Endpoints

Local &systemic *Infection*:

- Bacterial burden: colony forming units (CFU) per gram of tissue homogenate or ml of serum
- Incidence of infection (positive or negative culture from serum or tissue homogenate)

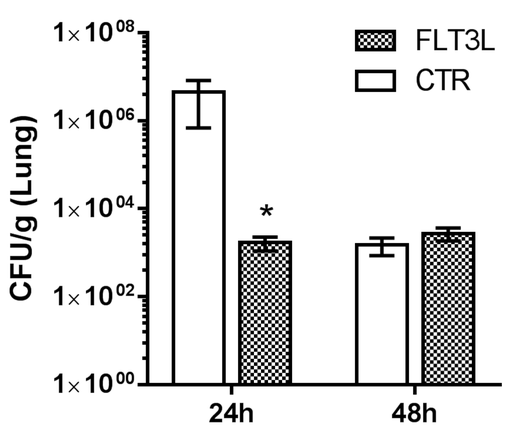
Local and systemic *Inflammation*:

- Interleukin 6 (IL-6) & Myeloperoxidase (MPO): ELISA from tissue homogenate or serum
- Histology: H&E preparation of lung, number of neutrophils & lymphocytes perialveolar and peribronchiolar: average number per 200x/400x high power field (HPF)

Survival (separate experiment): monitoring for 14 days post inoculation

Results

1. Improved Pulmonary Bacterial Clearance



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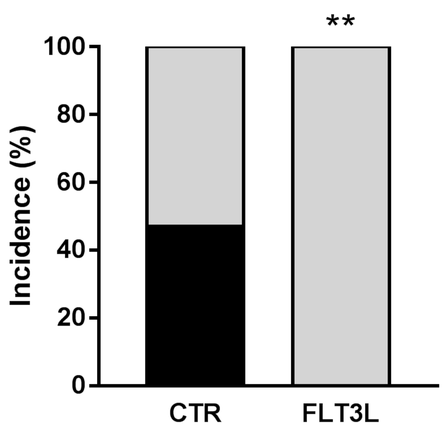

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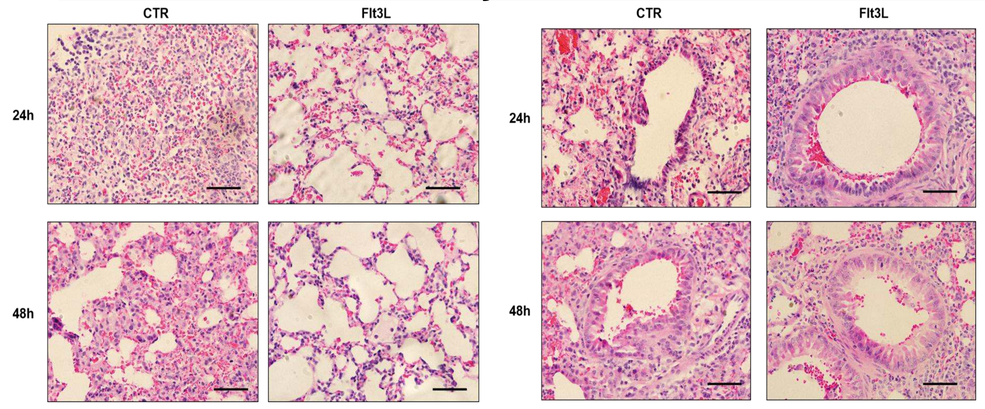
Figure 1: Bacterial burden of P. aeruginosa per gram of lung tissue at 24 and 48h p.i. (n=20, * : $p < 0.05$, 2-way ANOVA, Tukey's correction) ***Figure 2***: Incidence of severe lung infection (> 10^4 CFU/g) at 24h p.i. (n=15, ** : $p < 0.01$, Fisher's exact test)

2. Attenuated Pulmonary Inflammation and Immune Cell Infiltration



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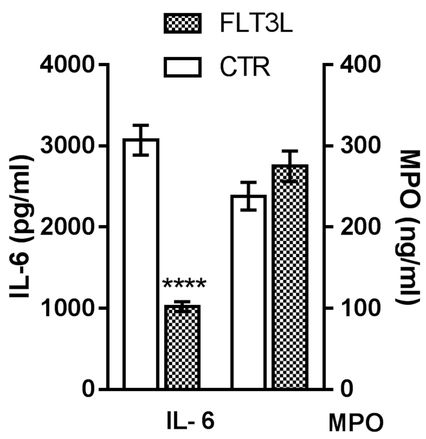

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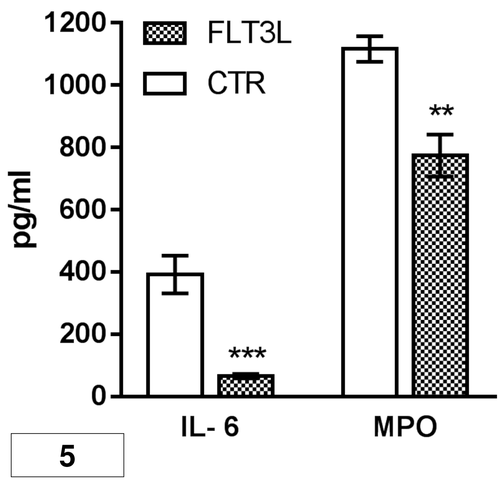
Figure 3:Representative photomicrographs of alveolar (left) and peribronchiolar (right) cellular infiltration and inflammation at 24 and 48h p.i. (Scale bar: 100µm; Mag: 400x) ***Figure 4:*** Levels of IL-6 and MPO in lung tissue at 24h p.i. (n=5, **** $p < 0.0001$, t-test)

3. Attenuated Systemic Inflammation and Bacteremia



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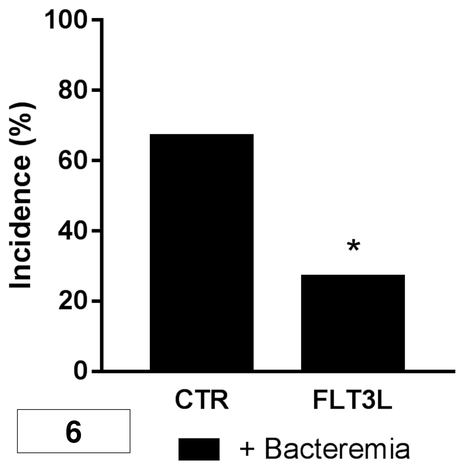

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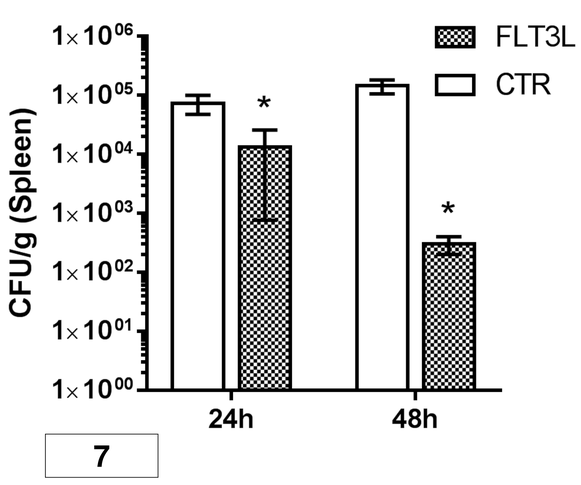
Figure 5: Levels of IL-6 and MPO in serum at 24h p.i. (n=15, ** $p < 0.01$; *** $p < 0.001$, 2-way ANOVA,Tukey) ***Figure 6***: Incidence of P. aeruginosa bacteremia (+, = positive blood culture) at 24h p.i. (n=15, * $p < 0.05$, Fisher's exact test)

4. Reduced Septic Distant Organ Manifestation



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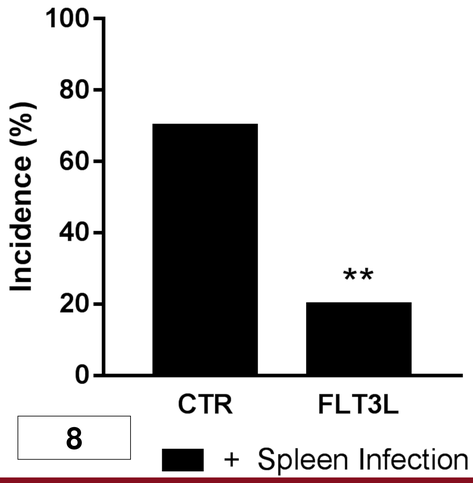

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Figure 7: bacterial burden per gram of spleen tissue at 24 and 48h p.i. (n=15, * $p < 0.05$, 2-way ANOVA,Tukey)
Figure 8: Incidence of distant organ manifestation (spleen) of P. aeruginosa infection 48h p.i. (n=20, ** $p < 0.01$, Fisher's exact test)

5. Comparable Survival and Delayed Mortality

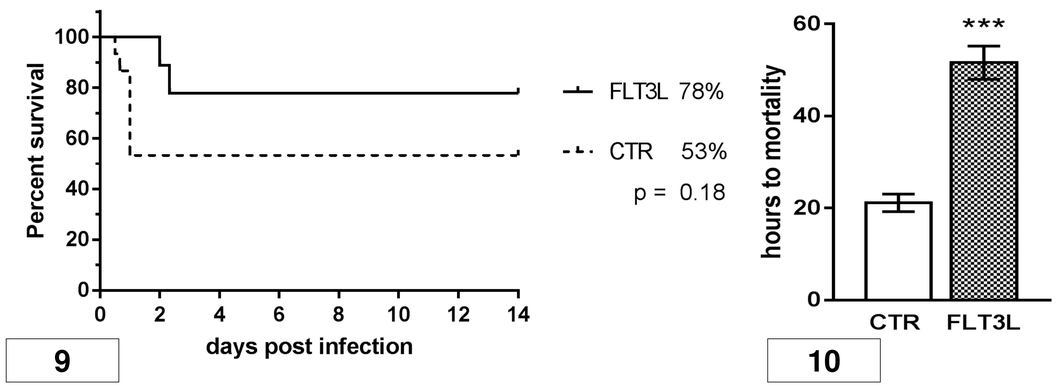

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Figure 9: 14 day survival after inoculation with P. aeruginosa and treatment with FLT3L or CTR (n=25, Logrank Mantel Cox test) ***Figure 10***: Time to mortality (hours) in expired animals (n=5, *** $p < 0.001$, t-test)

Conclusions

- **FLT3L** (fms-like tyrosine kinase 3 ligand)
  - ⇒Attenuated local infection and inflammation
  - ⇒Reduced systemic spread of infection and inflammation
  - ⇒Reduced septic manifestation of infection in distant organ
  - ⇒Did not increase mortality or inflammation
- **Potential clinical application: Initial prophylactic administration of Flt3L to**
  - ⇒boost innate and acquired immunity
  - ⇒reduce risk of infection and sepsis
  - ⇒augment antibiotic therapy against multi-drug-resistant pathogens

References

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Acknowledgements

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