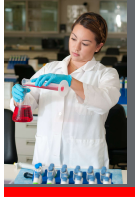

Immunopathogenesis of *Granulibacter bethesdensis,* an opportunistic pathogen causing recurrent infection in immunocompromised Patients

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Background

- Chronic Granulomatous Disease (CGD) is a primary immunodeficiency of phagocyte function due to defective NADPH oxidase resulting in a lack of reactive oxygen species (ROS) generation among innate immune cells such as neutrophils.
- ROS generation is a major antimicrobial defense mechanism of neutrophils and other phagocytic cells, and its impairment leads to susceptibility towards life-threatening pyogenic infections and inflammatory granulomas.
- *Granulibacter bethesdensis* (Gb) is a recently discovered bacterial pathogen known to cause recurrent and occasionally fatal infections in immune compromised CGD patients, owing to its ability to persist in a latent form in phagocytes.
- Very little is known regrading host immune responses required for efficient clearance of this pathogen in lung microenvironment, a primary site of recurrent infections in CGD patients.
- Using the NOX2-deficient mouse model of X-linked CGD (XCGD), we have established a pulmonary infection model of Gb infection to study immune cell functions, disease progression and infection outcome following intranasal infection with a clinical isolate of Gb.

Hypothesis

- A comparative Kinetic assessment of immune cell infiltrates in lungs of Gb infected wildtype (WT) and XCGD mice will provide important insights into detrimental and protective immune responses to help tailor future therapeutic strategies against this infection.

Experiment Methods

(A) Mice Studies

6–8-week-old mice were infected intranasally with 2x10^6 CFU of Gb and monitored for clinical signs of infection for up to 16 days.

Upon euthanasia, Blood, Bronchioalveolar lavage, and lungs were collected.

(B) Bronchioalveolar lavage fluid (BALF) isolation

(C) Preparation of cytospin slides

(D) Geimsa Staining

(E) Microscopy to determine cell counts.

Results

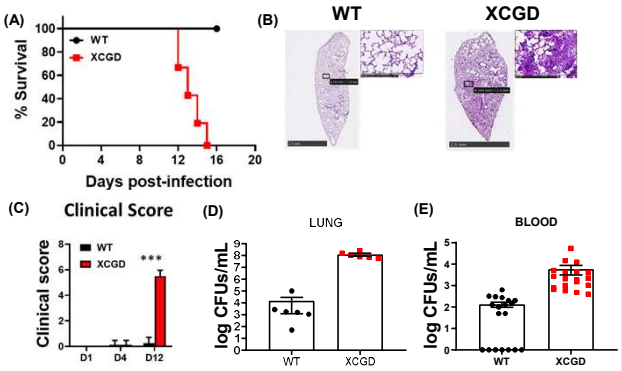


Figure 1: XCGD mice infected with Gb resulted in increased bacterial burden in lungs and blood leading to severe lung pathology. (A) Kaplan-Meyer survival analysis of Gb infection in WT and XCGD mice. (B) Lung tissues isolated from WT and XCGD mice upon euthanasia at Day 12-p.i. (C) The average clinical scores and the bacterial burden in (D) lungs and (E) blood are depicted as bar graphs. Paired T-test ($p<0.0001$)

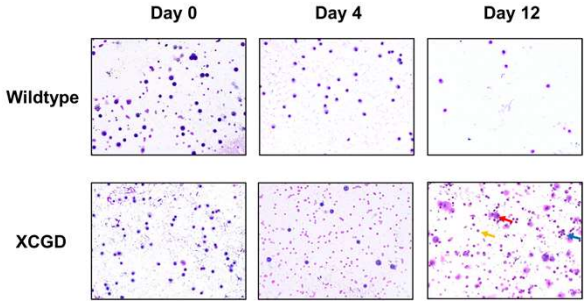
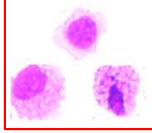
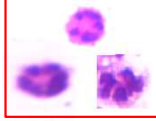


Figure 2: Wright-Giemsa staining of BALF cells representing different cell populations and their morphologies. Different colored arrows indicate the different types of nucleated cells identified. **Red: Macrophage/Monocyte**; **Orange: Neutrophil**; **Blue: Lymphocyte** (Giemsa, original magnification x200)

Identification of Different BAL cell types by Wright-Giemsa Staining



Macrophages & Monocytes
Macrophages and monocytes were identified as large cells with granular pattern across the cytoplasm. Tissue-resident macrophages differentiate from blood monocytes upon the presence of pro-inflammatory signals.



Neutrophils
Neutrophils are identified within tissues as a response infections. They perform a variety of functions to combat infection. These cells characteristically contain a multi-lobed nucleus that come in a variety of different shapes and sizes (poly-morpho-nuclear cells).

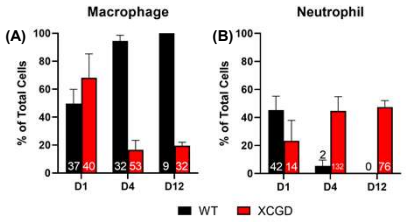


Figure 3: Bar graph showing the percentage of (A) macrophage and (B) neutrophils identified from BAL cells at Day 1, Day 4, and Day 12 p.i. The average numbers of cells counted within each frame are noted at the bottom of each bar for perspective.

Results & Conclusions

Compared to WT mice, XCGD mice were highly susceptible to infection and succumbed by day 12-14 post-infection. WT mice displayed a transient increase in clinical score correlating with moderate increase in local and systemic bacterial burdens characteristic of a mild, resolving infection. XCGD mice, on the other hand, exhibited a marked increase in clinical scores and bacterial burdens which remained high through Day 12-p.i., the time at which 100% of these mice became moribund.

Light Microscopy revealed that BAL cells primarily comprised of macrophages and neutrophils with the numbers mirroring the clinical severity of infection among WT and XCGD mice. We observed high numbers of macrophages and neutrophils at Day 1 among both groups of mice with the cell numbers returning to basal levels by Day 4 among WT mice. An increase in neutrophil counts was observed in XCGD mice at Day 4 and remained high at Day 12 indicating a continued state of inflammation within the lung microenvironment.

Future Directions

The rise of multi-drug resistant bacteria has forced us to reconsider the use of antibiotics. Shifting focus towards the use of alternative therapeutic strategies capable of boosting the immune system would strongly benefit immunocompromised patients inherently susceptible to severe infections. Understanding the key players responsible for triggering severe inflammatory responses and reducing their effects on resident cell populations would allow us to control the outcomes of debilitating infections.

References

1) Christine Deffert; Stephanie Carnesecchi; Huiping Yuan; Anne-Laure Rougemont; Tiina Kelkka; Rikard Holmdahl; Karl-Heinz Krause; Michela G Schäppi. Hyperinflammation of chronic granulomatous disease is abolished by NOX2 reconstitution in macrophages and dendritic cells. *JOP.* **2012.** 341-350.
2) D E Morgenstern; M A Gifford; L L Li; C M Doerschuk; M C Dinauer. Absence of respiratory burst in X-linked chronic granulomatous disease mice leads to abnormalities in both host defense and inflammatory response to Aspergillus fumigatus. *J Exp Med*. **1997.**
3) Jessica Chu; Margery G. Smelkinson; David W. Dorward; Kol A. Zarember; John I. Gallin. Early Intracellular Trafficking of *Granulibacter bethesdensis* in Human Macrophages. *ASM-II.* **2017.**
4) Atul Sharma; Katelyn McCann; Jitendra Kumar Tripathi; Pooja Chauhan; Christa Zerbe; Bibhuti B Mishra; Steven M Holland; Jyotika Sharma. Tamoxifen restores extracellular trap formation in neutrophils from patients with chronic granulomatous disease in a reactive oxygen species-independent manner. *J Allergy Clin Immunol.* **2019.**