IBCWG

20th Annual Meeting
April 27-30th, 2016
Blackwell Inn and Conference Center
Columbus, OH, USA

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International *Burkholderia cepacia* Working Group (IBCWG 2016)
The Blackwell Inn and Conference Center, The Ohio State University
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Wednesday, APRIL 27, 2016

4pm	**Registration opens, Pfahl 140**
6pm-8pm	**Welcome Reception, Pfahl 1st Floor Break Area** ***Appetizers/Refreshments/Beverages***
730pm-830pm	**Keynote Presentation, Pfahl 140** **Dr. Miguel Valvano, Queen's University Belfast** **"*Burkholderia cenocepacia*: the juggling act of surviving"**

Thursday, APRIL 28, 2016

8am-9am	**Registration, Pfahl 140** **Continental Breakfast, Pfahl 1st Floor Break Area and Pfahl 140**	
	Presentations in Pfahl 140	
9am	**Welcome: Dr. Amal Amer and Dr. Benjamin Kopp, The Ohio State University**	
910am-1030am	**Session 1.** **Biofilm busters: Interventions for *B. cenocepacia* biofilms** **Moderator:** Daniel Wozniak	
910am-935am	1.01	Heleen Van Acker **EXPLORING THE ROLE OF MINI-PROTEINS IN *BURKHOLDERIA CENOCEPACIA* BIOFILM FORMATION AND PERSISTENCE**
935am-1000am	1.02	Laura Novotny **STRUCTURAL STABILITY OF *BURKHOLDERIA CENOCEPACIA* BIOFILMS IS RELIANT ON eDNA STRUCTURE AND PRESENCE OF DNABII PROTEINS**
1000am-1025am	1.03	Connor Bowen **CIS-2-DODECENOIC ACID SIGNALLING MODULATES BIOFILM FORMATION AND ANTIBIOTIC RESISTANCE THROUGH THE TWO-COMPONENT SYSTEM BCAM0227/BCAM0228 IN *BURKHOLDERIA CENOCEPACIA*.**
1030am-1100am	**Break**	***Coffee/beverages/snacks***
11am-12pm	**Seminar – The post-genomic era: accelerating biological discovery through sequencing technology and population scale genomic analysis.** **Dr. Peter White, Nationwide Children's Hospital**	
12pm-1250pm	**Lunch, Ballroom A**	

100pm-230pm **Session 2. Novel Therapeutic Approaches**
Moderator: Benjamin Kopp

100pm-125pm 2.04 Kyle Caution
EPIGALLOCATECHIN-3-GALLATE (EGCG) INCREASES AUTOPHAGY EXPRESSION AND PROMOTES CLEARANCE OF *BURKHOLDERIA CENOCEPACIA*

125pm-150pm 2.05 Silvia Buroni
A NEW PROMISING BACTERICIDAL COMPOUND AGAINST *BURKHOLDERIA CENOCEPACIA*

150pm-215pm 2.06 Benjamin Kopp
CYSTEAMINE-MEDIATED CLEARANCE OF DRUG-RESISTANT PATHOGENS FROM CYSTIC FIBROSIS MACROPHAGES

230pm-300pm **Break** ***Beverages/Snacks***

300pm-430pm **Session 3. Genomic secrets of *Burkholderia* infections and inflammation**
Moderator: Leo Eberl

300pm-325pm 3.07 Mikhail Gavrilin
INDUCTION OF MONOCYTE/MACROPHAGE INFLAMMATORY PATHWAYS BY *B. CENOCEPACIA*

325pm-350pm 3.08 Geoffrey L. Winsor
THE *BURKHOLDERIA* GENOME DATABASE: MORE GENOMES, MORE ANALYSES, MORE PLANS

350pm-415pm 3.09 Steven Higgins
THE ESSENTIAL GENOME OF *BURKHOLDERIA CENOCEPACIA* H111

415pm-430pm **Next IBCWG planning**

600pm **Group dinner at Z Cucina. Bus will start loading at 600pm from the Blackwell for 630 dinner.**

Friday, APRIL 29, 2016

8am-9am **Continental Breakfast, Pfahl 1st Floor Break Area/Pfahl 140**
Presentations in Pfahl 140

9am-1030am **Session 4. Systems analysis of *Burkholderia* infections**
Moderator: Deborah Yoder-Himes

900am-925am 4.10 Tapas Mal
METABOLOMICS PROFILING OF CYSTIC FIBROSIS MACROPHAGES

925am-950am 4.11 Cerith Jones
SPECIES-WIDE ANALYSIS OF *BURKHOLDERIA GLADIOLI* BIOACTIVE SECONDARY METABOLITES

950am-1015am 4.12 John Varga
TN-SEQ ANALYSIS REVEALS PUTATIVE ESSENTIAL GENES IN *BURKHOLDERIA CENOCEPACIA* CHRONIC GRANULOMATOUS DISEASE MOUSE INFECTIONS.

1030am-1100am **Break** ***Coffee/beverages***

1100am-1215pm **Session 5. Regulatory and selective forces in *Burkholderia* infections**
Moderator: Vaughn Cooper

1100am-1125am 5.13 Vaughn S. Cooper
LONG-TERM EVOLUTION OF BURKHOLDERIA MULTIVORANS DURING A CHRONIC CYSTIC FIBROSIS INFECTION REVEALS SHIFTING FORCES OF SELECTION

1125am-1150am 5.14 José Degrossi
EXPLORING β-LACTAMASES IN *BURKHOLDERIA CONTAMINANS*

1150am-1215pm 5.15 Andrea M. Sass
IDENTIFICATION OF SMALL REGULATORY RNAS IN *BURKHOLDERIA CENOCEPACIA*

1215pm-1pm **Lunch, Ballroom A**

200pm **Group Tour of Columbus. Bus will pick up from the Blackwell at 200pm**
630pm **Dinner on your own**

Saturday, APRIL 30, 2016

8am-9am **Continental Breakfast, Pfahl 1st Floor Break Area/Pfahl 140**
Presentations in Pfahl 140

900am-1030am **Session 6. Dilemmas of *dolosa*: *Burkholderia dolosa* pathogenicity**
Moderator: Greg Priebe

900am-925am 6.16 Bradley Clark
THE EFFECTS OF MUTATIONS IN THE TOLQRAB COMPLEX OF *BURKHOLDERIA DOLOSA*

925am-950am 6.17 Matthew M. Schaefers
A SINGLE AMINO ACID CHANGE IN *BURKHOLDERIA DOLOSA* FIXL ALTERS FIX ACTIVITY AND PATHOGENICITY

950am-1015am 6.18 Deborah Yoder-Himes
THE HOST IMMUNE RESPONSE TO *BURKHOLDERIA DOLOSA*

1030am-1100 am **Break** ***Coffee/beverages***

1100am-1230 pm **Session 7. Interplay of virulence and immunity**
Moderator: Amal Amer

1100am-1125am **7.19** Silvia Buroni
NEW ANTIVIRULENCE COMPOUNDS AFFECTING *BURKHOLDERIA CENOCEPACIA* QUORUM SENSING *IN VITRO* AND *IN VIVO*

1125am-1150am **7.20** Kathrin Krause
CASPASE-11 CONTRIBUTES TO BURKHOLDERIA CENOCEPACIA CLEARANCE IN MICE

1150am-1215pm **7.21** Frank Robledo-Avila
CYSTIC FIBROSIS NEUTROPHILS HAVE COMPROMISED ROS/NETS RESPONSE AND CANNOT CONTROL *BURKHOLDERIA CENOCEPACIA* INFECTION

1215pm **Closing Remarks**

1230pm **Lunch, Ballroom B**

ABSTRACTS

1.01 EXPLORING THE ROLE OF MINI-PROTEINS IN *BURKHOLDERIA CENOCEPACIA* BIOFILM FORMATION AND PERSISTENCE

Heleen Van Acker, Aurelie Watty, Tom Coenye

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Burkholderia cenocepacia J2315 is a member of the *Burkholderia cepacia* complex (*Bcc*), a group of opportunistic pathogens that can cause severe lung infections in cystic fibrosis patients. Infections are often difficult to treat due to resistance, biofilm formation and persistence. *Bcc* bacteria have large multi-replicon genomes (6-9 Mb) and the function of a large fraction of genes annotated as "hypothetical" or "conserved hypothetical" is still unknown. While previous research has predominantly focussed on larger proteins, evidence is accumulating that genes encoding polypeptides with a length smaller than 100-200 amino acids are ubiquitous in the genomes of all living organisms and are involved in various biological processes. The goal of the present study is to elucidate the role of mini-proteins in *B. cenocepacia* biofilm formation and persistence.
We focused on genes smaller than 300 nucleotides of which the function is unknown. Almost 10 % (646) of the genes in the *B. cenocepacia* genome are smaller than 300 nucleotides and more than half of these are annotated as hypothetical proteins. For 234 of them no similarity could be found with non-hypothetical genes in other bacteria using BLAST (E < 10-5 and identity > 40 %). However, based on available transcriptomic data the majority of these genes were found to be up- or downregulated in stress conditions (treatment with tobramycin, H_2O_2 or chlorhexidine, low oxygen, low pH, low iron or heat). Using available RNA sequencing data, a list of 27 mini-proteins highly expressed in biofilms (RPKM > 200) was compiled and those are currently being studied more in detail. Preliminary results indicate that overexpression of some of these mini-proteins leads to an increase in biofilm biomass. Flow cytometry was used to measure fluorescence in translational GFP reporter fusion mutants. For several mini-proteins the expression was higher after treatment with Tob, Cip or H_2O_2.
In conclusion our results confirm that mini-proteins are present in the genome of *B. cenocepacia* J2315 and indicate that they are involved in various biological processes.

1.02 STRUCTURAL STABILITY OF *BURKHOLDERIA CENOCEPACIA* BIOFILMS IS RELIANT ON eDNA STRUCTURE AND PRESENCE OF DNABII PROTEINS

Laura A. Novotny[1], Amal O. Amer[2], Steven D. Goodman[1] and Lauren O. Bakaletz[1]

[1]Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital and The Ohio State University College of Medicine, Columbus, Ohio, USA
[2]Department of Microbial Infection and Immunity, Center for Microbial Interface Biology, The Ohio State University, Columbus, Ohio, USA

Contact: Laura Novotny (laura.novotny@nationwidechildrens.org)

Cystic fibrosis (CF) is the most common lethal inherited genetic disorder affecting Caucasians. Even with medical advances, CF is life-shortening with patients typically surviving only to age 38. Infection of the CF lung by *Burkholderia cenocepacia* presents exceptional challenges to medical management of these patients as clinically this microbe is resistant to virtually all antibiotics, is highly transmissible and infection of CF patients with this microbe renders them ineligible for lung transplant, often the last lifesaving option. Here we have targeted two abundant components of the *B. cenocepacia* biofilm for immune intervention: extracellular DNA and DNABII proteins, the latter of which are bacterial nucleic acid binding proteins. Treatment of *B. cenocepacia* biofilms with antiserum directed at one of these DNABII proteins (integration host factor or IHF) resulted in significant disruption of the biofilm *in vitro*. Moreover, when anti-IHF mediated destabilization of a *B. cenocepacia* biofilm was combined with exposure to traditional antibiotics, *B. cenocepacia* resident within the biofilm, typically highly resistant to the action of antibiotics, were now rendered susceptible to killing. Epitope mapping of IHF revealed functional and immunoprotective domains within the DNA-binding tip regions of each subunit of the IHF heterodimer. To precisely target these regions, and further develop antibodies against IHF as a novel therapeutic, we generated monoclonal antibodies (MAbs) against specific 20-mer portions within both subunits of the DNA-binding tip and non-DNA-binding tail regions (the latter of which served as negative controls). *In vitro*, treatment with tip-specific MAbs resulted in a >65% reduction in biomass of biofilms formed by *B. cenocepacia* strain K56. Tail-specific MAbs were ineffective, as anticipated. Collectively, these findings support further development of strategies that target DNABII proteins as a novel approach for treatment of CF patients, particularly those whose lungs are infected with *B. cenocepacia*.

1.03 CIS-2-DODECENOIC ACID SIGNALLING MODULATES BIOFILM FORMATION AND ANTIBIOTIC RESISTANCE THROUGH THE TWO-COMPONENT SYSTEM BCAM0227/BCAM0228 IN *BURKHOLDERIA CENOCEPACIA*.

Connor Bowen[1], Robert P. Ryan[1]

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Burkholderia cenocepacia is an opportunistic human pathogen that uses cis-2-dodecenoic acid (BDSF) a signal molecule of the diffusible signal factor (DSF) family to control virulence factor expression and biofilm formation. The BDSF signal synthase, BCAM0581, catalyzes the production of BDSF signal, which is an analogue of the signal DSF (*cis*-11-methyl-2-dodecenoic acid), originally identified in the plant bacterial pathogen *Xanthomonas campestris* pv. *campestris.* Two sensors for BDSF have now been described in *B. cenocepacia*; BCAM0580, which is designated RpfR, and the sensor kinase BCAM0227. RpfR is comprised of an N-terminal PAS domain and GGDEF and EAL domains. GGDEF and EAL domains are implicated in the synthesis and degradation, respectively, of the second messenger cyclic di-GMP. Perception of BDSF by RpfR sharply enhances its second messenger cyclic-di-GMP phosphodiesterase activity and consequently causes a reduction in the intracellular level of the nucleotide in *B. cenocepacia*, which consequently affects a range of biological activities. While BCAM0227 is a complex sensor kinase, predicted to have two transmembrane helices and a large periplasmic loop of 300 amino acids. Comparative transcriptome analysis showed that BCAM0227 is involved in regulation of a subset of functions that are controlled by BDSF in *B*. *cenocepacia*. Unlike RpfR, signal transduction beyond BCAM0227 is poorly understood. Here we have used a mutagenesis, transcriptional analysis and phosphotransfer analysis to characterize BCAM0227 potential cognate regulator BCAM0228. Transcriptome profiling analysis established the scope of the BAM0228 'regulon' and demonstrated that the regulator controls expression of extensive set of these genes controlled by BCAM0227. Mutation of BCAM0227 and *BCAM0228* gave rise to similar changes in biofilm formation and antibiotic resistance suggesting a genetic link. Importantly, BCAM0227 has the ability to phosphorylate BCAM0228 *in vitro*. Taken together, these findings identify BCAM0228 as a cognate regulator of BCAM0227 and a potential target for interference in virulence-related signaling in *B. cenocepacia*.

2.04 EPIGALLOCATECHIN-3-GALLATE (EGCG) INCREASES AUTOPHAGY EXPRESSION AND PROMOTES CLEARANCE OF *BURKHOLDERIA CENOCEPACIA*

Kyle Caution[1], Mia Tazi[1], Kathrin Krause[1], Hany Khalil[2], Daniel Layman[1], Abdulmuti Saleh[1], Estelle-Cormet Boyaka[3], Amal O. Amer[1]

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Contact: Kyle Caution; kyle.caution@osumc.edu

Cystic Fibrosis (CF) is a lethal, inherited disease that affects ~70,000 children and adults worldwide. 85% of deaths are a result of progressive and destructive pulmonary inflammation exacerbated by bacterial infection. Human and murine CF airway epithelial and macrophages are defective in the physiological and highly regulated process called autophagy. This process is crucial for natural turnover of damaged proteins and organelles and also protective against infection and disease. Ineffective autophagy fails to degrade the dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) protein that aggregates in the cells of CF patients. This deficiency also fails to clear bacterial colonization in the CF lung, aggravating an already robust inflammatory response. *Burkholderia cenocepacia* (*B.c.*) infection is one of the most feared CF pathogen, as it expresses an innate antibacterial resistance to most therapies and can readily be transmitted through direct patient contact. Infection and colonization of the CF lung by *B.c.* can result in a disease state characterized by accelerated loss of lung function, sepsis, and death called "cepacia syndrome". Previously, we have shown that functional autophagy is key to the host immune response against *B.c.* There is a crucial need to foster novel strategies that will improve the health of CF patients infected with *B.c.*, but effective therapies are lacking. Epigallocatechin-3-gallate (EGCG) is a natural catechin found in tea extracts that exhibits antioxidant and anti-inflammatory properties. It has also been shown to inhibit DNA methyltransferases, blocking the methylation of DNA at the epigenetic level. We have demonstrated that human and murine CF-derived macrophages (expressing the F508del mutation) have lowered autophagy gene expression contributing to significantly elevated *B.c.* replication and inflammation. Therefore, we hypothesize that defective autophagy in human and murine CF cells is due to increased methylation of autophagy genes, thus decreasing expression of essential autophagy proteins, facilitating persistence of *B.c.* infection and intensifying inflammation within the CF lung. Furthermore, we propose that treatment with EGCG will increase expression of key autophagy players, promoting clearance of the pathogen and decreasing inflammation and pulmonary destruction. Our data demonstrate for the first time that autophagy genes are hypermethylated in CF macrophages compared to WT cells, at the basal level. When treated with EGCG, autophagy molecules were upregulated in CF macrophages when compared to cells treated with vehicle. Our study found that *B.c.* infection hypermethylated autophagy genes, while at the same time promoted downregulation of autophagy molecules. Notably, treatment of CF macrophages after infection with *B.c.* partially restored autophagy protein expression when compared to non-treated infected cells. *In vivo* treatment with EGCG promoted significant restriction of *B.c.* in the lungs of CF mice after 48h infection. EGCG treatment also significantly inhibited the dissemination of the pathogen to the liver and spleen. Analysis of autophagy protein expression also showed increased autophagy molecule expression upon EGCG treatment *in vivo*. All together, these data demonstrate that epigenetic regulation of autophagy genes and the modulation of their expression by EGCG play a crucial role in the pathophysiology in CF.

2.05 A NEW PROMISING BACTERICIDAL COMPOUND AGAINST *BURKHOLDERIA CENOCEPACIA*

Silvia Buroni[1], April S. Gislason[2], Viola C. Scoffone[1], Maria Silvina Stietz[2], Laurent R. Chiarelli[1], Alberto Azzalin[3], Vadim Makarov[4], Silvia T. Cardona[2,5], and Giovanna Riccardi[1]

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Contact: Silvia Buroni, silvia.buroni@unipv.it

In the struggle against *Burkholderia cenocepacia* it is essential to find new antimicrobial compounds to fight infections caused by drug-resistant strains affecting cystic fibrosis patients in order to improve their life expectancy.
Here we present a new promising bactericidal drug belonging to the 2,1,3-benzothiadiazol-5-yl family (10126109), with an MIC of 8 µg/ml against *B. cenocepacia*. The compound is active against clinical isolates and other members of the Bcc, as well as against other Gram-negative and -positive bacteria.
By a chemogenomic approach and super resolution microscopy, the mechanism of action of 10126109 was shown to rely on the inhibition of the cell division apparatus. Further biochemical and genetic approaches suggested that the compound targets the essential cytoskeletal cell division protein FtsZ. In particular, the compound was demonstrated to inhibit the GTPase and polymerization activities of FtsZ *in vitro* at micromolar concentrations. Toxicity experiments carried out on CF epithelial bronchial cells and *Caenorhabditis elegans* revealed low toxicity of the compound. Evaluation of antibiotic effect *in vivo* is being performed in *C. elegans* infected with *B. cenocepacia*. New derivatives of 10126109 will be synthesized with the aim to find more active molecules. Moreover, inhalable formulations will be developed in order to administer it to mice to evaluate its *in vivo* efficacy.

2.06 CYSTEAMINE-MEDIATED CLEARANCE OF DRUG-RESISTANT PATHOGENS FROM CYSTIC FIBROSIS MACROPHAGES

Kaivon Assani[1], Hannah Rinehardt[1], Chandra Shrestha[1], and Benjamin Kopp[1,3]

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Contact/Presenter: Benjamin Kopp, Benjamin.kopp@nationwidechildrens.org

Members of the *Burkholderia cepacia* complex are virulent, multi-drug resistant pathogens that survive and replicate intracellularly in patients with cystic fibrosis (CF). We have discovered that *B. cenocepacia* cannot be cleared from CF macrophages due to defective autophagy, causing continued systemic inflammation and infection. Additionally, defective autophagy has a feedback inhibition of oxidative killing in CF macrophages. Defective autophagy in CF is mediated through inflammatory activation of transglutaminase-2 (TG2), which causes the accumulation of autophagy initiating proteins such as Beclin-1 and the cargo marker p62. Cysteamine is an inhibitor of TG2 and a proteostasis regulator with the potential to restore autophagy. Therefore, we undertook a study of cysteamine's effects on CF macrophage function. Peripheral blood monocyte derived macrophages (MDMs) were isolated from CF and non-CF donors. A THP-1 monocyte cell line was used with a CFTR inhibitor to verify experimental results. Alveolar macrophages were isolated from the bronchoalveolar lavage of CF patients. Macrophages were infected with *B. cenocepacia* clinical isolate strain k56-2 and *B. multivorans* strain FC-445. Cysteamine causes direct bacterial killing of live *B. cenocepacia* and *B. multivorans* in the absence of cells. Additionally, *B. cenocepacia* growth was significantly decreased in CF MDMs treated with cysteamine compared to standard antibiotics alone. Cysteamine decreases p62 and Beclin-1 accumulation in CF, leading to increased *Burkholderia* packaging into autophagosomes as seen by co-localization with the autophagosome marker LC3 in both alveolar and peripheral blood macrophages. Finally, cysteamine enhances bacterial killing via increased NADPH oxidase formation in CF, leading to decreased persistent ROS production as bacterial are killed. In summary, we have shown that cysteamine is able to clear *Burkholderia* from CF macrophages by inhibiting TG2, thereby reducing the accumulation of p62, restoring Beclin-1, and thus re-establishing autophagy in CF macrophages. Cysteamine may be an effective adjunct to antibiotic regimens in CF.

3.07 INDUCTION OF MONOCYTE/MACROPHAGE INFLAMMATORY PATHWAYS BY *B. CENOCEPACIA*

Mikhail Gavrilin, M.D. Wewers, A.O. Amer
Late abstract, full information to follow.

Alejandra Bosch was a late cancellation, her abstract is below

THE PRESENCE OF GENES ASSOCIATED TO THE GENOMIC ISLAND *BCENGI11* ARE HIGHLY PREVALENT IN *BURKHOLDERIA CONTAMINANS* ISOLATES RECOVERED DURING CHRONIC LUNG INFECTION IN PATIENTS WITH CYSTIC FIBROSIS

Mariana Leguizamón[1], Marisa Bettiol[2], María J. Palau[2], Patricia Montanaro[3], Laura Cazzola[4], Silvia Perez[4]; Claudia Prieto[1], Pablo Martina[4], Florencia Vignolles[1], Beltina León[1], Osvaldo Yantorno[1] and Alejandra Bosch[1*]

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Among the *Burkholderia cepacia* complex species, *B. contaminans* represents the most frequently recovered from patients with cystic fibrosis (CF) in Argentina (1). Although for some patients *B. contaminans* respiratory tract infection is transient, its acquisition most typically results in a chronic infection with a gradual decline in lung function (2). It was recently reported for *B. cenocepacia* J2315 (belonging to the ET12 lineage) that the "*Burkholderia cepacia* epidemic strain marker" (BCESM) genomic region located on the genomic island (GI), BcenGI11, contains an operon (BCAM0257-8-9) possible involved in persistence, quorum sensing expression, and virulence (3).
We here aimed to study a possible relation between persistence and establishment of *B. contaminans* chronic infection, and the presence of genes associated to the genomic island BcenGI11, which was not reported for this species, so far. For this purpose, we analyzed 145 *B. contaminans* isolates recovered from 52 patients attended in 3 different CF reference centers of Argentina, between 2004-2015. The BCESM and the BCAM0257-8-9 regions were detected by PCR -using *B. cenocepacia* specific primers- and sequenced. Persister cells in 24-h old biofilms exposed to ciprofloxacin concentrations of 4 × the MIC was quantified, and QS signals expression was analyzed by means of AHLs biosensors.
Our results showed that the 94.7 % of the chronic infected patients (36 out of 38) and the 28.6 % of the patients with transient infections (4 out of 14 patients) harbored both the BCESM and the BCAM257-9 regions. Sequence analysis showed that the amplification products of these regions had an identity of 93 and 95 % with those of *B. cenocepacia* J2315, respectively. The percentage of surviving persisters was between 5 and 10 % for *B. contaminans* isolates carrying the operon in contrast to less than 2% for isolates for which did not. QS signals expression was also positively correlated the presence of the BCESM genomic region. These results showed that, the presence of the operon contained in the BCESM region might be responsible for the difficulties in the eradications of *B. contaminans* chronic infection.

3.08 THE BURKHOLDERIA GENOME DATABASE: MORE GENOMES, MORE ANALYSES, MORE PLANS

Geoffrey L. Winsor[1], Bhavjinder K. Dhillon[1], Claire Bertelli[1], James E. Zlosnik[2], Silvia T. Cardona[3,4] and Fiona S.L. Brinkman[1]

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Advances in whole genome sequencing have the potential to generate hundreds to thousands of genome assemblies based on strains isolated from outbreaks or other clinical and environmental studies. In 2015, we released a new version of the Burkholderia Genome Database with an expanded ability to integrate sequence data, annotations and metadata for thousands of complete and draft *Burkholderia* genomes. While the database has grown in size, it continues to place an emphasis on high quality genome annotation updates through regular review of the scientific literature and community-based efforts to annotate gene and protein function, subcellular localization, virulence factors and drug targets. These annotations can be downloaded in formats (e.g. GFF3, GTF and GAF 2.0) suitable for downstream analysis using external software tools.

We are currently integrating results from computational analyses that identify antimicrobial resistance (AMR) genes and assess their association with genomic islands (GIs) versus non-GIs while identifying AMRs that are over-represented in *Burkholderia* genomes. Analyses associated with the identification of putative drug targets, vaccine candidates and pathogen-associated genes as well as tools that map orthologs between strains have also been integrated. Visualization of alternate genome annotations and transcriptome data has been enhanced by addition of the JBrowse genome browser and views of recently improved genomic island predictions in IslandViewer have been incorporated.

As part of our future plans, we wish to capitalize on the success of a related *Pseudomonas* community-based annotation initiative and organize a similar community-driven effort to annotate high quality Gene Ontology (GO) terms and associated evidence codes in select *B. cenocepacia* strains. Annotations resulting from this initiative will facilitate sophisticated queries, data exchange, gene functional enrichment analysis while providing increased confidence and transparency. In collaboration with other members of the International *Burkholderia cepacia* Working Group, we've established a website at http://ibcwg.org to help with this goal and foster community sharing of information and resources relevant to *Burkholderia cepacia* complex researchers. These collective changes are only the beginning of the development of further enhancements to aid whole-genome comparative studies, pan-genome analysis, and more population-based analyses, as we further expand the database in an era of thousands of genomes.

3.09 THE ESSENTIAL GENOME OF *BURKHOLDERIA CENOCEPACIA* H111

Steven Higgins, Maria Sanchez-Contreras, Marta Pinto, Aurelien Carlier and Leo Eberl

Department of Plant and Microbial Biology, University of Zürich, Zollikerstrasse 107, CH-8008, Zürich.

Contact: Steven Higgins

The study of the minimum set of genes required to sustain life is a fundamental question in biological research. Recent studies on bacterial essential genes suggested that between 350 and 700 genes are essential to support autonomous bacterial cell growth. Essential genes are considered to be of interest as potential new antimicrobial drug targets and hence, our aim was to identify the essential genome of the opportunistic pathogen *Burkholderia cenocepacia* H111.

Using a Tn-Seq protocol we identified the minimal essential genome in an environment where most nutrients are supplied (LB) and compared this with a minimal medium environment with citrate as a carbon source.

B. cenocepacia has a 7.7MB multi replicon genome which is predicted to encode around 7500 genes. Our analysis suggests that the minimal essential genome consists of between 600 and 700 genes, which is only around 10% of the predicted ORF's of this bacterium. As expected a further 100 to 150 genes were required to support growth in minimal media. Many of the essential genes encode core metabolic processes involved in the biosynthesis of amino acids and nucleotides. In addition genes involved with energy generation, cell division and transcription / translation appear to be indispensable.

Oxygen is limited in biofilms and steep oxygen gradients have been reported in the CF lung. To analyze the essential genes in a micro-oxic environment our Tn-Seq protocol was repeated in 0.5% O_2 to determine the low oxygen regulon of *B. cenocepacia* H111.

The majority of the essential genes were found on chromosome 1. The essential genes on chromosome 2 were predominately clustered together in one region of the chromosome. Interestingly, these genes are always found on chromosome 2 in the genus *Burkholderia* but are coded on chromosome 1 in members of the closely related genus *Ralstonia*. It is tempting to speculate that the transfer of these essential genes to chromosome 2 in an ancestral *Burkholderia* species was a key event for the phylogenetic separation of the two genera.

4.10 METABOLOMICS PROFILING OF CYSTIC FIBROSIS MACROPHAGES

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Cystic Fibrosis (CF) is a hereditary genetic disorder, most commonly found in the Caucasian population. It is caused by mutations present in both copies of the cystic fibrosis transmembrane conductance regulator (CFTR), and is inherited in the autosomal recessive fashion. The CFTR is involved in mucus production, as well as the secretion of sweat and digestive enzymes. Patients with CF are prone to respiratory complications not only due to a thickening of mucus lining the lungs, but also due to defective innate immune response to pathogens. As a result, CF patients are susceptible to lung infection. One of the common bacterial infections found in the lungs of a CF patient is *Burkholderia cenocepacia* (Bc). In this study, we have used macrophages isolated from a CF mouse model with a mutation in the delF508-CFTR. Similar to a human patient with CF, cells from this mouse model with this mutation produce mutant CFTR protein missing a phenylalanine residue, leading to misfolding of the protein and thus reduction in CFTR function. Wild type (WT) macrophages are used as a control. The aim of this study is to determine differences in the metabolomics profile of uninfected and Bc infected macrophages with and without CF mutation. We have obtained metabolomics profile of macrophages with and without infection by employing state-of-the-art high resolution Nuclear Magnetic Resonance spectroscopy (NMR). Significant differences were observed in the metabolomics profiles between WT and CF non-infected macrophages, and macrophages infected with Bc. Our data offer a distinctive metabolomics profile for an important pathogen that will help advance our understanding of microbial pathogenesis in CF. In addition, a distinctive inherent metabolomics signature for CF macrophages sheds light on the wide effect of CFTR dysfunction.

4.11 SPECIES-WIDE ANALYSIS OF *BURKHOLDERIA GLADIOLI* BIOACTIVE SECONDARY METABOLITES

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Members of the diverse *Burkholderia* genus are known to produce bioactive secondary metabolites, including the enacyloxins produced by *Burkholderia ambifaria* [1]. As part of wider screen for compounds with potential as novel antibiotics it was observed that *Burkholderia gladioli* strongly inhibits the growth of clinically important pathogens. Organisms from this species have previously been shown to produce a respiratory toxin, bongkrekic acid [2], and the bright yellow phytotoxin toxoflavin [3].

Solvent extraction of growth media followed by Flash Chromatography has revealed the production of multiple bioactive compounds by *B. gladioli* BCC238. This isolate produces a novel macrolide antibiotic, Gladiolin. The structure of Gladiolin has been elucidated and the gene cluster responsible for its synthesis confirmed by insertional mutagenesis. The remaining bioactivity of *B. gladioli* BCC238 can be attributed to Toxoflavin production.

A collection of 88 *B. gladioli* isolates were genome re-sequenced using Illumina technology and encoded biosynthetic gene clusters identified using AntiSMASH [4]. Total biosynthetic capability varied across *B. gladioli,* with the Toxoflavin gene cluster conserved in all isolates, while the Gladiolin cluster was found in a subset of 34 genomes.

A phylogeny-driven screen of *B. gladioli* bioactivity is underway to uncover the full potential of *B. gladioli* as an antibiotic producer. In summary, the phenotypic and genotypic evidence suggests that *B. gladioli* is worthy of further follow up as a novel antibiotic producing microorganism.

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4.12 TN-SEQ ANALYSIS REVEALS PUTATIVE ESSENTIAL GENES IN *BURKHOLDERIA CENOCEPACIA* CHRONIC GRANULOMATOUS DISEASE MOUSE INFECTIONS.

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The *Burkholderia cepacia* complex is a group of at least 18 distinct species of bacteria known in part for their ability to cause disease in a variety of immunocompromised people, including those with cystic fibrosis (CF) or chronic granulomatous disease (CGD). CGD is a hereditary disease that causes a deficiency in reactive oxygen species (ROS) in the immune system. We used a *mariner*-based transposon to produce of a library of approximately 300,000 mutants. This library was used to perform two Tn-Seq experiments with *Burkholderia cenocepacia* strain J2315 to identify potential virulence factors and assess gene essentiality in a CGD mouse model of infection. Mice were inoculated via intratrachael infection, and transposon insertion frequencies were measured in the input population, and from recovered bacterial pools from the lung, liver, and spleen.

Based on comparisons with insertion frequencies determined through a Monte Carlo simulation, we identified 131 genes with at least a 64-fold decrease in insertion frequency in both input libraries; however, these are primarily artifacts due to the presence of multicopy genes. There were an additional 555 genes whose frequencies were reduced between 8- and 32-fold in both input samples compared to the simulation. Known essential genes, such as *dnaE*, *gyrB*, and the thiamine biosynthesis cluster, fell into this category suggesting that it represents our limit of detection in these experiments.

When we compared results from the lung, liver, and spleen to the input results, we did not observe any mutants with 64-fold reductions. In the lung, liver, and spleen we observed 22, 130, and 240 mutants that were reduced 8- to 32-fold compared to the input population, respectively. In the lung, these included mutants for tryptophan biosynthesis and the *afc* gene cluster on chromosome III. Mutants reduced in the liver additionally included other biosynthetic pathways, several type IV pilus components, and DNA binding proteins. From the spleen, mutants in numerous metabolic and cell surface proteins were also reduced.

Our data suggest that during growth in LB there is a reduction in population frequency of many mutants indicating that they are essential during *in vitro* growth. Compared to the input pool, there were many fewer mutants that showed similar reductions in the lungs; the liver and spleen had 5- and 10-times more mutants that showed this level of reduction, respectively, suggesting that those organs impart a much broader selection on the mutant population compared to the lung. These data can serve to inform future work on genes essential for niche survival, for virulence factor characterization, and drug development

5.13 LONG-TERM EVOLUTION OF *BURKHOLDERIA MULTIVORANS* DURING A CHRONIC CYSTIC FIBROSIS INFECTION REVEALS SHIFTING FORCES OF SELECTION

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Burkholderia multivorans is an opportunistic pathogen capable of causing severe disease in patients with cystic fibrosis (CF). Patients may be chronically infected for years, during which the bacterial population evolves in response to unknown forces. Here we analyze the genomic and functional evolution of a *B. multivorans* infection that was sequentially sampled from a CF patient over 20 years. The population diversified into at least four primary, coexisting clades with distinct evolutionary dynamics. The average substitution rate was only 2.4 mutations/year but notably some lineages evolved more slowly whereas one diversified more rapidly by mostly nonsynonymous mutations. Ten loci, mostly involved in gene expression regulation and lipid metabolism, acquired three or more independent mutations and define likely targets of selection. Further, a broad range of phenotypes changed in association with the evolved mutations, including antimicrobial resistance, biofilm regulation, and the presentation of lipopolysaccharide O-antigen repeats that was directly caused by evolved mutations. Additionally, early isolates acquired mutations in genes involved in c-di-GMP metabolism that associated with increased c-di-GMP intracellular levels. Accordingly, these isolates showed lower motility, increased biofilm and adhesion to CFBE41o- epithelial cells, each important trait for bacterial persistence. The timing of the emergence of this clade of more adherent genotypes correlated with the period of greatest decline in patient´s lung function. Altogether, our observations suggest that selection on *B. multivorans* populations during long-term colonization of the CF lung either directly or indirectly targets adherence, metabolism, and changes in the cell envelope related to adaptation to the biofilm lifestyle.

5.14 EXPLORING β-LACTAMASES IN *BURKHOLDERIA CONTAMINANS*

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Antibiotic-resistant bacteria pose a serious threat to human health, and the increase in infections caused by such pathogens is a growing concern worldwide. Resistance mediated by β-lactamases is widely distributed among bacteria. Although different antibiotic resistance mechanisms have been described in the *Burkholderia cepacia* complex (Bcc) and β-lactamases have been reported, the role of these enzymes is poorly understood in Bcc species.
The aim of this study was to identify and to perform a first approach in the characterization of β-lactamases present in *B. contaminans*, the most frequent Bcc species isolated in Argentina.
Bioinformatic analysis using different tools (BLAST, ARTEMIS, RAST and ResFinder) of *B contaminans* genomes available in public databases, revealed at least five putative β- lactamases genes. Sequence comparison of β-lactamases found in FFH2055 - strain isolated from a CF patient in Argentina- and other Bcc species displayed identities ranged from 82% to 100%.
Class A β-lactamase CTX-M and class D β-lactamase BlaD genes were selected for further studies. Distribution of both genes among our collection of *B. contaminans* isolates was analyzed. Furthermore, genes were cloned and expressed into *E. coli* to investigate antibiotic resistance activity.
Results indicate that these genes are not present in all strains used in this study displaying differences between clinical and industrial isolates. Slight activity against cefotaxime, ceftazidime and ceftoxitin was detected in transformed *E. coli*.
These results provide insight into the presence of β-lactamases in *B. contaminans* suggesting a secondary role in the antibiotic resistance of these bacteria that could act as reservoirs of β-lactamases to be transferred to other species.

5.15 IDENTIFICATION OF SMALL REGULATORY RNAS IN *BURKHOLDERIA CENOCEPACIA*

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In recent years, an increasing number of small non-coding RNAs have been discovered in prokaryotes. Non-coding RNAs regulate gene expression, either by binding to other RNAs or by binding to proteins. Non-coding RNAs often target regulatory elements and thus fine-tune the respective regulatory processes. Among the regulatory cascades influenced by non-coding RNAs are global developmental processes such as biofilm formation.
In *Burkholderia* species, non-coding RNAs are to date largely uncharacterised. To identify small non-coding RNAs expressed in *Burkholderia cenocepacia* biofilms, transcription start sites within its genome were mapped by differential RNA sequencing [1]. Transcription start sites located in intergenic regions and not associated with a coding sequence were further examined for presence of candidate regulatory small RNAs. Short transcripts with a pronounced secondary structure, a rho-independent terminator and a relatively high degree of conservation were further analysed. Expression of candidate small RNAs in different growth phases, stress conditions and in biofilms was analysed by qPCR and Northern Blotting. Putative targets of small RNAs were predicted with CopraRNA [2]. Four candidate small regulatory RNAs were selected for deletion, based on results of RNA-silencing and on whether their predicted targets and their genome context showed any similarity with known small RNAs of other bacteria.
One small RNA-deletion mutant (delta-nc35) showed increased susceptibility to carbapenems, NaOCl, and envelope stress (SDS), increased resistance to salt stress, increased flocculation and a change in biofilm morphology.

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6.16 THE EFFECTS OF MUTATIONS IN THE TOLQRAB COMPLEX OF *BURKHOLDERIA DOLOSA*

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Burkholderia dolosa was responsible for an outbreak in the cystic fibrosis clinic at Children's Hospital Boston from 1998 to 2005 that resulted in the colonization of over 40 patients. *B. dolosa* is not a common CF isolate nor is it commonly isolated from other natural environments. The genome of the outbreak strain AU0158 was sequenced and found to contain several genes encoding potential virulence factors not commonly found in other Bcc members. It is not known why this particular strain caused an outbreak. To examine this strain and species further, we conducted a transposon mutant library screen to identify genes essential for colonization in the host lung and for septic dissemination. One operon essential for lung colonization contained the genes encoding the TolQRAB-Pal-CpoB outer membrane complex. This complex is believed to be involved in membrane integrity in other species, but its specific cellular role is still unclear. We created strains bearing mutations in *tolQ*, *tolB*, and *cpoB* and tested them in a variety of phenotypic test to determine if mutations in these genes resembled their effects in other species based on published literature. Similar to *tolQ* mutants in other species, our *B. dolosa* mutants exhibit a filamentous phenotype under standard and low salt growth conditions while appearing to grow at a rate consistent with wild-type *in vitro* based on spectrophotometry. These mutant strains show a defect in serum resistance which is consistent with its effects in other bacterial species. Our mutant strains also show defects in resistance to many antibiotics including β-lactams. These results together indicate that the *B. dolosa* TolQRAB-Pal-CpoB complex is important for membrane integrity and may be a good target for therapeutics.

6.17 A SINGLE AMINO ACID CHANGE IN *BURKHOLDERIA DOLOSA* FIXL ALTERS FIX ACTIVITY AND PATHOGENICITY

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Background. While investigating the genomic diversity of 112 isolates of the *Burkholderia cepacia* complex member *B. dolosa* collected over 16 years from an outbreak in cystic fibrosis patients, we identified *fixL* (BDAG_01161) as a gene showing signs of strong positive selection. This gene has homology to *fixL* of the rhizobial FixL/FixJ two-component system. FixL is a sensory histidine kinase that detects oxygen tension and phosphorylates the transcription factor FixJ in low oxygen, leading to activation of the *fixK* gene. Our previous work identified that the *fixLJ* system is involved in biofilm formation, motility, and persistence in a murine model. Interestingly the reference strain, *B. dolosa* AU0158, which was isolated from the index patient after several years of colonization, contains a mutation in the *fixL* gene compared to the ancestral isolate. In this study we sought to understand the implications of this mutation in *fix*-mediated phenotypes.
Methods. We constructed a *fixLJ* deletion mutant of *B. dolosa* strain AU0158 using allelic replacement as well as isogenic strains complementing the deletion mutant with either S439 *fixL* (reference sequence in strain AU0158) or W439 *fixL* (ancestral sequence) and *fixJ* (reference sequence). We measured swimming motility as well as the ability of these constructs to form biofilm on PVC plates. We also measured the oxygen-dependent activation of a *B. dolosa fixK* promoter carried by the constructs using a beta-galactosidase reporter assay. In a murine model of pneumonia after intranasal inoculation, we assessed the ability of these *B. dolosa* strains to persist in the lung and disseminate to the spleen.
Results. *B. dolosa* carrying the ancestral FixL sequence (W439) produced significantly (p value <0.001) more biofilm than isogenic bacteria carrying the reference *fixL* sequence (S439). Interestingly, *B. dolosa* carrying the ancestral *fixL* were non-motile while *B. dolosa* carrying the reference sequence were motile. *B. dolosa* carrying the ancestral *fixL* showed 2- to 4-fold higher activation of *fixK* transcription than the reference *fixL* sequence when grown in both full and limited oxygen environments (p values <0.05). In the murine pneumonia model, *B. dolosa* carrying the ancestral *fixL* sequence had 8- to 10-fold lower viable bacteria in the lungs and spleen 7 days post infection compared to *B. dolosa* carrying the reference *fixL* sequence (p values <0.05).
Conclusion. *B. dolosa* carrying a FixL point mutation arising later during human infection is less able to form biofilms, more motile, less able to induce *fixK* transcription, and better able to persist *in vivo* in the murine lung and spleen compared to *B. dolosa* carrying the ancestral genotype.
Funding: Cystic Fibrosis Foundation

6.18 THE HOST IMMUNE RESPONSE TO *BURKHOLDERIA DOLOSA*

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Burkholderia dolosa caused an outbreak at Children's Hospital Boston from 1998 to 2005. Over 40 patients contracted *B. dolosa* and many died due to complications from this organism. However, the index patient and several others were colonized with this strain and failed to developed symptoms. How *B. dolosa* (and other Bcc members) can remain in the host lung without being eliminated by the immune response remains unclear. We examined the interaction of *B. dolosa* and mammalian lungs using murine lung models and cultured cells. Our previous results indicate that *B. dolosa* can remain in the lung tissue far longer than another CF pathogen, *Pseudomonas aeruginosa*, and it also recruits fewer immune cells. Further, we previously found that the production of 15 different cytokines in mouse lungs was very low for *B. dolosa* which indicates that *B. dolosa* is either not sensed by the host immune cells or that it is actively shutting down the host immune response. To test between these two hypotheses, we used cultured murine cells and primary human bone marrow-derived cells to determine if these cells can recognize *B. dolosa* in the absence of an intact immune response. In all cases, the cultured cells can readily sense *B. dolosa* and produce a robust cytokine response. Interestingly, this strong response does not depend on the flagellar status of *B. dolosa*. Together with our previous data, these results suggest that *B. dolosa* is more likely actively shutting down the host immune response in order to remain inside the host lung. This could contribute to why human CF patients can remain colonized by *B. dolosa* for such long periods of time.

7.19 NEW ANTIVIRULENCE COMPOUNDS AFFECTING *BURKHOLDERIA CENOCEPACIA* QUORUM SENSING *IN VITRO* AND *IN VIVO*

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Finding new molecules able to inhibit *Burkholderia cenocepacia* virulence in the host is of primary importance, together with the discovery of new antimicrobials.
In this way, interfering with the activity of quorum sensing (QS) synthases may render the bacteria unable to produce virulence factors, making bacteria less able to colonize the host. Also, as therapies directed at inhibiting QS do not directly kill the bacteria the development of resistance is less likely.
Here, new antivirulence molecules able to inhibit the QS synthase CepI have been developed. Among a series of 7 different compounds assayed against recombinant CepI two (10826023 and 11526011) were found to be effective inhibitors of the enzyme, with IC_{50} values within micromolar range. These compounds have been shown to interfere with the protease and siderophore production of *B. cenocepacia*, and with its ability to form biofilm. Also, the survival of *Caenorhabditis elegans* infected with *B. cenocepacia* was greatly improved after treatment with the compounds, thus indicating a good *in vivo* activity against *Burkholderia* virulence. Finally, the two molecules are not toxic for human cells. These anti-virulence drugs could potentially be used in combination with established or novel antimicrobials to improve the current therapy.

7.20 CASPASE-11 CONTRIBUTES TO BURKHOLDERIA CENOCEPACIA CLEARANCE IN MICE

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The intracellular protease caspase-11 directly binds cytosolic LPS derived from gram-negative bacteria. As part of the non-canonical inflammasome caspase-11 promotes Nlrp3-dependent processing of caspase-1 in response to various bacteria entering the host cytosol thereby leading to cytokine secretion and cell death. Beyond that, caspase-11 stimulates the fusion of phagosomes harboring *Legionella pneumophila* with lysosomes by modulating the actin cytoskeleton. Nevertheless, the role of caspase-11 regarding vacuolar bacteria remains poorly defined. *B. cenocepacia* causes severe infections in patients with pulmonary diseases like cystic fibrosis (CF) or chronic granulomatous disease. Maturation and acidification of the *B. cenocepacia* phagosome in CF macrophages is delayed and allows survival of the organism within the vacuolar compartment whereas wild-type macrophages efficiently clear the infection. Because *B. cenocepacia* harbors a natural resistance to most antibiotics it is hard to eradicate from colonized lung tissue. Understanding the restriction of *B. cenocepacia* within immunocompetent host cells will be important to develop new treatment strategies for infected patients.

In this study we demonstrate that caspase-11 deficiency leads to increased replication of *B. cenocepacia* in murine macrophages and infected lungs accompanied by bacterial dissemination to liver and spleen. Furthermore, colocalization of *B. cenocepacia* with lysosomes or the autophagy marker LC3 is reduced in caspase-$11^{-/-}$ macrophages. Autophagy activity upon starvation measured by LC3 puncta formation is considerably lower in caspase-11 deficient macrophages as well. Moreover, caspase-$11^{-/-}$ macrophages exhibit less colocalization of *B. cenocepacia* with the actin-binding protein cofilin. Together our data demonstrate that caspase-11 is required for trafficking of the *B. cenocepacia*-containing vacuole thereby promoting bacterial clearance.

7.21 CYSTIC FIBROSIS NEUTROPHILS HAVE COMPROMISED ROS/NETS RESPONSE AND CANNOT CONTROL *BURKHOLDERIA CENOCEPACIA* INFECTION

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Cystic Fibrosis (CF), one of the most common genetic diseases worldwide, is caused by defects in the ion channel CF transmembrane conductance regulator (CFTR). CF patients are highly susceptible to specific infections such as *Burkholderia cenocepacia* (*B. cenocepacia*), which may cause excessive lung inflammation, tissue damage, and fatal loss of pulmonary function. *B. cenocepacia* infection in CF patients induces the recruitment of large amount of neutrophils into the lungs, which release toxic proteins contained in their primary granules, aggravating the lung tissue damage. We hypothesized that CF neutrophils are intrinsically deficient in their oxidative functions and therefore, unable to control the infection by *B. cenocepacia*. Purified CF bone marrow neutrophils and CF human neutrophils were infected with *B. cenocepacia* to measure the release of reactive oxygen species (ROS) and the assessment of ROS dependent bacterial killing. We found that both, mouse and human, CF neutrophils were unable to release ROS and this correlated with a reduced capacity in killing bacteria, as compared to wild type or healthy donor neutrophils. Because ROS production is proposed to regulate the formation of neutrophils extracellular traps (NETs), which is considered one of the most important antimicrobial mechanisms in neutrophils, we then infected CF neutrophils with *B. cenocepacia* to quantify NETs. We found that CF neutrophils were deficient in developing NET structures. Together, our findings demonstrate that CF neutrophils have an intrinsic defect in the production of ROS, and consequently, the antimicrobial mechanism dependent of ROS pathway, resulting in a diminished capacity to control *B. cenocepacia* infection.

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