Colorado Mycobacteria Conference 2022:

Focus on Nontuberculous Mycobacteria

May 31, 2022 – June 3, 2022

Colorado State University, Lory Student Center
Fort Collins, CO, USA

Scientific organizers:
Charles L. Daley (National Jewish Health, Denver)
Barbara E. Laughon (NIAID, Bethesda)
Anil K. Ojha (New York State Department of Health, Wadsworth Center, Albany)
Dean C. Crick (Colorado State University, Fort Collins)
Mary C. Jackson (Colorado State University, Fort Collins)

Symposium on the Environmental Risks for Nontuberculous Mycobacterial Lung Infections

June 4, 2022

Scientific Organizers: D. Rebecca Prevots (NIAID, Bethesda, MD); Rachel Thomson (University of Queensland, Brisbane, Australia); Joseph O. Falkinham (Virginia Tech, Blacksburg, VA); Ettie Lipner (NIAID, Bethesda; MD); Jennifer Honda (National Jewish Health, Denver, CO); Joshua French (University of Colorado, Denver); Maura Donohue (United States Environmental Protection Agency, Cincinnati, OH)

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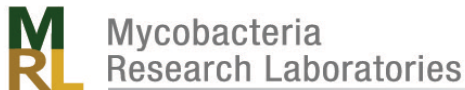

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Kia Ferrel (School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia; Tuberculosis Research Program, Centenary Institute, Sydney, Australia)
Ju Mi Lee (Department of Microbiology, Institute for Immunology and Immunological Disease, Graduate School of Medical science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Republic of Korea)

Colorado Mycobacteria Conference 2022: Focus on NTM

Tuesday May 31 – Friday June 3, 2022

Agenda

Please scan with a QR code reader on your smartphone

PROGRAM

Colorado Mycobacteria Conference 2022: Focus on NTM
Tuesday May 31 – Friday June 3, 2022

Tuesday May 31, 2022 [5:00 – 9:00 pm]

5:00 – 6:30 pm Registration desk opens, packet pickup

5:00 – 6:30 pm Welcome Cocktails and Hot Appetizers

Welcome and Keynote Lectures

Discussion Leader: Charles L. Daley (National Jewish Health)

6:30 – 6:45 pm: Patrick Brennan (Colorado State University, Fort Collins, CO) – *Welcome Address*

6:45 – 7:20 pm: Anne E. O'Donnell (Georgetown University, Washington D.C.) – *"Clinical overview of NTM lung infections"*

7:20 – 7:50 pm Break

7:50 – 8:20 pm: Joseph O. Falkinham, III (Virginia Tech, Blacksburg, VA)
– *"Nontuberculous Mycobacteria in the Environment"*

Wednesday June 1, 2022

Session 1: Epidemiology and Clinical Aspects of NTM Diseases

Discussion Leader: Jerry A. Nick (National Jewish Health, Denver, CO)

8:00 – 8:05 am: Introduction by Discussion Leader

8:05 – 8:35 am: Rebecca Prevots (NIH/NIAID, Bethesda, MD) – *"Epidemiology of NTM in the US and Globally"*

8:35 – 9:05 am: Stacey Martiniano (Children's Hospital Colorado, Aurora, CO) – *""Clinical manifestations of NTM infection with a focus on the cystic fibrosis population"*

9:10 – 9:40 am: Amy Leitman (President; NTMir, Miami, FL) – *"NTM Lung Disease: Patient Preferences and Experiences"*

9:40 – 10:00 am: Heather R. Jordan (Mississippi State University, Starkville, MS) – *"Mycobacterium ulcerans: Tracking transmission"*

10:00 – 10:30 am Coffee Break

Session 2: Diagnostics and Biomarkers

Discussion Leader: Delphi Chatterjee (Colorado State University, Fort Collins, CO)

10:30 – 11 am: Jerry A. Nick (National Jewish Health, Denver, CO) – *"Culture-independent markers of NTM lung disease"*

11 – 11:40 am: Short talks selected from abstracts:

- 11 – 11:20 am: Lucas Boeck (University of Basel, Basel, Switzerland) – *"Mycobacterium abscessus killing profiles reveal clinical outcomes independent of drug resistance"*
- 11:20 – 11:40 am: Richard Robinson (The Ohio State University, Columbus, OH) – *"Immune biogeography of nontuberculous mycobacteria (NTM) infected airways in people with cystic fibrosis (PwCF)"*

11:40 am – 12:10 pm: Jakko van Ingen (Radboud University Medical Center, The Netherlands) – *"Drug susceptibility testing of NTM – academic exercise or key biomarker?"*

12:10 – 1:30 pm Lunch Break

Session 3: Genetics and Physiology of NTM

Discussion Leader: Mary Jackson (Colorado State University, Fort Collins, CO)

1:30 – 1:35 pm: Introduction by Discussion Leader

1:35 – 2:05 pm: Luiz Pedro de Carvalho (Francis Crick Institute, London, United Kingdom) – *"Macro-evolution as a source of unknown antibiotic resistance determinants"*

2:05 – 3:25 pm: Short talks selected from abstracts:

- 2:05 -2:25 pm: Chidiebere Akusobi (Harvard Medical School, Boston, MA) – *"High-density transposon mutagenesis in Mycobacterium abscessus identifies an essential penicillin-binding lipo-protein (PBP-lipo) involved in septal peptidoglycan synthesis and antibiotic sensitivity"*
- 2:25 – 2:45 pm: Rebecca Davidson (National Jewish Health, Denver, CO) – *"Lineage analysis of Mycobacterium abscessus subsp. abscessus isolates from a treatment refractory infection reveals genomic adaptations over a five-year period"*
- 2:45 – 3:05 pm: Brittany Ross (Georgia Institute of Technology, Atlanta, GA) – *"Identification of molecular determinants that govern morphotype-specific physiology in Mycobacteroides abscessus"*
- 3:05 – 3:25 pm: Scarlet Shell (Worcester Polytechnic Institute, Worcester, MA) – *"The sRNA B11 controls virulence-associated phenotypes in Mycobacteroides abscessus"*

3:30 – 6:00 pm Poster Session & Light Appetizers

Thursday June 2, 2022

Session 4: Pathogenic Strategies of NTM and Host Immune Response to NTM Infections

Discussion leaders: Mercedes Gonzalez-Juarrero (Colorado State University, Fort Collins, CO); Anil K. Ojha (New York State Department of Health, Wadsworth Center, Albany, NY)

8:00 – 8:05 am: Introduction by Discussion Leader (Bacteriology)

8:05 – 8:35 am: Andres Floto (University of Cambridge, Cambridge, United Kingdom) – *"Understanding how Mycobacterium abscessus causes disease"*

8:35 – 9:05 am: Laurent Kremer (Institut de Recherche en Infectiologie de Montpellier; CNRS UMR9004, France) – *"The virulence determinants of Mycobacterium abscessus infection in zebrafish"*

9:05 – 9:25 am: Short talk selected from abstracts:

- Nicola Lore (IRCCS Ospedale San Raffaele, Milan, Italy) – *"Unravelling the pathogenicity of Mycobacterium abscessus clinical isolates in CF pulmonary epithelial cell and mouse models of respiratory infection"*

9:25 – 9:55 am: Laurent Marsollier (Université d'Angers – INSERM, Angers, France) – *"New insight on Buruli ulcer physiopathology"*

9:55 – 10:25 am Coffee Break

10:25 – 10:30 am: Introduction by Discussion Leader (Immunology)

10:30 – 11:00 am: Luiz Bermudez (Oregon State University, Corvallis, OR) – *"Strategies of Mycobacterium avium and Mycobacterium abscessus to Initiate Airway Infections"*

11:00 – 11:30 am: Céline Cougoule (IPBS – Toulouse; CNRS-Université Toulouse III, France) – *"Human airway organoids for Cystic Fibrosis-driven Mycobacterium abscessus infection modelling"*

11:30 – 11:50 am: Short talk selected from abstracts:

- Haleigh Gilliland (Michigan State University, East Lansing, MI) – *"Defining complex mechanistic interactions and responses by macrophages during Mycobacterium abscessus infection"*

11:50 am – 2:00 pm Lunch Break

2:00 – 2:30 pm: Marcela Henao-Tamayo (Colorado State University, Fort Collins, CO) – *"NTM mucosal-induced immune response has an enhanced protective effect on BCG's efficacy against Mycobacterium tuberculosis infection"*

2:30 – 3:10 pm: Short talks selected from abstracts:

- 2:30 – 2:50 pm: Elsjee Pienaar (Purdue University, West Lafayette, IN) – *"An Agent-Based Model to Assess the Contribution of Menopause-Associated Immunological Changes to Increased Risk of MAC Infection"*
- 2:50 pm – 3:10 pm: Susan Baldwin (Seattle Children's Research Institute, Seattle, WA) – *"Immunogenicity and protection against Mycobacterium avium with a heterologous RNA prime and protein boost vaccine regimen"*

3:15 – 5:00 pm Poster session

6:00 – 9:00 pm Gala Dinner at Coopersmith's (Pool Side)

Friday June 3, 2022

Session 5: Intervention Strategies and Clinical Trials

Discussion leaders: Tiffany Burnett (Cystic Fibrosis Foundation, Bethesda, MD), Barbara E. Laughon (NIH/NIAID, Bethesda, MD)

8:00 – 8:05 am: Introduction by Discussion Leaders
8:05 – 8:35 am: Martin I. Voskuil (University of Colorado Anschutz School of Medicine, Aurora, CO) – *"RS ratio and supporting physiologic assays for mycobacterial drug and regimen evaluation"*
8:35 – 9:05 am: Rebekah M. Dedrick (Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA) – *"Therapeutic potential of phages for mycobacterial infections"*
9:05 – 9:25 am Short talk selected from abstracts:

- Winifred Akwani (University of Surrey, Surrey, United Kindom) – *"Understanding antibiotic penetration into mycobacterial biofilms using NanoSIMS"*

9:25 – 9:50 am Coffee Break

9:50 – 10:20 am: Thomas Dick (Hackensack Meridian Health, Nutley, NJ) – *"Rifamycins for M. abscessus lung disease"*

10:20 – 11:00 am Short talks selected from abstracts:

- 10:20 -10:40 am: Jickky Palmae (Hackensack Meridian Health, Nutley, NJ) – *"Mycobacterium tuberculosis DprE1 inhibitor OPC-167832 is active against Mycobacterium abscessus"*
- 10:40 – 11 am: Timothy Shaw (Queen's University Belfast, Belfast, United Kingdom) – *"Human mesenchymal stromal cells inhibit Mycobacterium avium growth in in vitro and in vivo models of pulmonary infection*"

11:00 – 11:30 am Richard E. Lee (St Jude Children's Research Hospital, Memphis, TN) – *"Lessons learned from drugging M. abscessus with spectinomycin analogs"*

11:30 – 1:00 pm Lunch Break

1:00 – 1:30 pm: Kenneth N. Olivier (NIH/NHLBI, Bethesda, MD) – *"NTM trial dilemma: Outcomes assessment in rare disease setting – Who, what, when and how to measure"*

1:30 – 2:10 pm: Short talks selected from abstracts:

- 1:30 – 1:50 pm: Jason Holder (Endolytix Technology, Beverly, MA) – *"Shredding Intracellular NTMs with a Macrophage-Targeted Enzymatic Cocktail"*
- 1:50 – 2:10 pm: Andreanne Lupien (McGill International TB Center, Montreal, Canada) – *"The arylvinylpiperazine amide AX-35 targets the cytochrome bc_1 in Mycobacterium abscessus"*

2:15 pm Presentation of Poster Awards

2:30 pm Closing Remarks

Environmental Risks for Nontuberculous Mycobacterial Lung Infections Symposium

Saturday June 4, 2022 [8:00 am – 3:00 pm]

Session 1: Environmental Sampling, Household Sampling, and Laboratory Techniques

Discussion leader: Noah Fierer (University of Colorado); Max Salfinger

8:00 – 8:10 am: Rebecca Prevots: Welcome and Introduction

8:10 – 8:30 am: Matthew Gebert (University of Colorado) – *"Predicting the environmental preferences of NTM"*

8:30 – 8:50 am: Jennifer R. Honda (National Jewish Health) – *"Guess what? We can see you! – Environmental NTM in man-made and natural sites of Hawai'i"*

8:50 – 9:10 am: Jerry Cangelosi (University of Washington) – *"NTM Exposures and Sources of Infection in Home Environments"*

9:10 – 9:30 am: Janet Stout (Special Pathogens Laboratory) – *"Improved Environmental Isolation of NTM: Field and Laboratory Results."*

9:30 – 9:50 am: Ted Marras (University Health Network, Canada) – *"Water quality attributes and pulmonary NTM infection in Ontario, Canada"*

10:00 – 10:25 am Coffee Break

Session 2: Epidemiologic and Statistical Approaches for Estimating Environmental and Climatic Risks for NTM

Discussion leader: Rebecca Prevots; Rachel Mercaldo (NIAID)

10:25 – 10:30 am: Introduction by Discussion Leader

10:30 – 10:50 am: Rachel Thomson (University of Queensland, Australia) – *"Geospatial analysis of NTM risk in Queensland, Australia"*

10:50 – 11:10 am: Ettie Lipner (NIAID) – *"Water quality risk factors for NTM infection among US CF patients"*

11:10 – 11:30 am: Kozo Morimoto (Fukujuji Hospital, Japan) – *"NTM and the environment—Japanese perspectives on home and natural environments"*

11:30 – 11:50 am: Joshua French (University of Colorado) – *"Spatial scan methods for disease cluster detection"*

12:00 am – 1:30 pm Lunch and Poster Session (Moderator: Julia Marshall)

1:30 – 2:10 pm Panel Discussion 1: Sampling and culturing methods for NTM
Discussion moderators: Noah Fierer; Max Salfinger

2:10 – 2:20 Break

2:20 – 3:00 pm Panel Discussion 2: Statistical and analytic challenges in estimating NTM risk
Discussion moderators: Joshua French; Rachel Mercado

SHORT TALK and POSTER PRESENTERS

Colorado Mycobacteria Conference 2022:

Focus on NTM

May 31- June 3, 2022

Symposium on Environmental Risks for Nontuberculous Mycobacterial Lung infections

June 4, 2022

Presenter	Affiliation	Poster #
Abdelaziz, Rana	Institut für Pharmazie, Martin-Luther-Universität Halle- Wittenberg, Halle (Saale), Germany	66
Ahmed, Sahiba	Burnett School of Biomedical Sciences at the University of Central Florida College of Medicine, Orlando, USA	29
Akusobi, Chidiebere	Harvard T.H. Chan School of Public Health, Boston, USA. Harvard Medical School, Boston, USA	33
Akwani, Winifred	National Physical Laboratory, National Centre of Excellence in Mass Spectrometry Imaging (NiCE- MSI), Teddington, Middlesex, United Kingdom. Department of Microbial Sciences, School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Surrey, United Kingdom	83
Amin, Anita	Colorado State University, Fort Collins, USA	13
Ayerakwa, Eunice	West African Centre for Cell Biology of Infectious Pathogen, University of Ghanas,, Accra, Ghana	44
Baker, Brett	Microbion Corporation, Bozeman, USA	82
Baldwin, Susan	Seattle Children's Research Institute, Seattle, USA	57
Bar Oz, Michal	Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel	1
Barkan, Daniel	Hebrew University, Rehovot, Israel	16
Belardinelli, Juan	Colorado State University, Fort Collins, USA	72
Beuchel, Andreas	Institut für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany	62
Boeck, Lucas	Department of Biomedicine, University of Basel, Basel, Switzerland	14
Bolden, Nicholas	University of Pennsylvania Perelman School of Medicine, Philadelphia, USA. Children's Hospital of Philadelphia, Philadelphia, USA	28
Bonefont, Lauren	University of Central Florida, Orlando, USA	26

Presenter	Affiliation	Poster #
Calado Nogueira de Moura, Vinicius	National Jewish Health, Denver, USA	7
Calhoun, Kara	University of Colorado Denver, Denver, USA	12
Chhabra, Avneet	Phigenics, LLC, Reno, USA	90
Crist, Matthew	Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA, USA	3
Davidson, Rebecca M.	National Jewish Health, Denver, USA	6,9,34
Dawrs, Stephanie N.	National Jewish Health, Denver, USA	49
De Boeck, Nathan	VIB-KU Leuven Center for Microbiology, Leuven, Belgium	22
De, Kavita	Colorado State University, Fort Collins, USA	23
Dutt, Taru	Colorado State University, Fort Collins, USA	47
Eskandarian, Haig Alexander	Lawrence Berkeley National Laboratory, Berkeley, USA. University of California, San Francisco, San Francisco, USA	43
Ferrell, Kia	Sydney Institute for Infectious Diseases and the Charles Perkins Centre, The University of Sydney, Camperdown, NSW, Australia. Tuberculosis Research Program, Centenary Institute, Sydney, Australia. School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia	40
Foulon, Mélanie	Department of Biochemistry, Faculty of Science, University of Geneva, Geneva, Switzerland	42
Funck, Tobias	Division of Clinical Tropical Medicine, Centre of Infectious Diseases, Heidelberg University Hospital, Heidelberg, Germany. Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, USA	27
GHARBI, Rim	INSTITUT PASTEUR DE TUNIS, TUNIS, Tunisia	37
Gilliland, Haleigh	Michigan State University, East Lansing, USA	55

Presenter	Affiliation	Poster #
Guiewi, Gaelle	Trudeau Institute, Saranac Lake, USA	81
Gupta, Rashmi	University of Central Florida, Orlando, USA	25
Hardman, Michelle	Department of Life Sciences, Manchester Metropolitan University, Manchester, United Kingdom	51
Hasan, Nabeeh	National Jewish Health, Denver, CO, USA. National Jewish Health, Denver, CO, USA	92,93
Hendrick, Haley A.	University of Hawai'i, Honolulu, USA	91
Hendrix, Jo	National Jewish Health, Denver, USA	32
Hobbie, Sven N.	University of Zurich, Zurich, Switzerland	68
Holder, Jason	Endolytix Technology, Beverly, USA	86
Honda, Jennifer R.	National Jewish Health, Denver, USA	46
Hunt Serracin, Augusto	University of Texas at Arlington, Arlington, USA	18
Jang, Jichan	Gyeongsang National University, Jinju, Korea, Republic of	70
Lamichhane, Gyanu	Johns Hopkins University, Baltimore, USA. Johns Hopkins University, Baltimore, USA	21,71
Lan, Tian	University of Minnesota, Minneapolis, USA	61
Lang, Markus	Martin-Luther-Universität, Halle (Saale), Germany	59
Lee, Ju Mi	Department of Microbiology, Institute for Immunology and Immunological Disease, Graduate School of Medical science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea, Republic of. Department of Microbiology, Institute for Immunology and Immunological Disease, Graduate School of Medical science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea, Republic of	74,75
Lee, Sunhee	Duke University, Durham, USA. University of Texas Medical Branch, Galveston, USA	45

Presenter	Affiliation	Poster #
Lian, Elena	Colorado State University, Fort Collins, USA	24
Lore, Nicola	Emerging Bacterial Pathogens Unit, IRCCS Ospedale San Raffaele, Milan, Italy	54
Lupien, Andréanne	McGill International TB Centre, Montréal, Canada. Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre, Montréal, Canada. Global Health Institute, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland	87
Maloney, Sara	RTI International, Durham, USA	64
Mann, Lea	Institut für Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany	63
Marion, Estelle	Inserm, Angers, France	4
Maruyama, Fumito	Hiroshima University, Higashi-Hiroshima, Japan	88
Mason, Clifford	Crestone, Inc, Boulder, USA	77
Matriz, Jobel	National Institutes of Health, Bethesda, USA	48
McGowen, Kerry	Harvard University, Boston, USA	30
McManus, William	University of Notre Dame, Notre Dame, IN, USA	89
Mejia-Chew, Carlos	Washington University, Saint Louis, USA	2
Munsiff, Sonal	University of Rochester School of Medicine and Dentistry, Rochester, USA	5
Murphy, Patricia A.	St. Jude Children's Research Hospital, Memphis, USA	78
Naor, Noga	Hebrew University of Jerusalem, Rehovot, Israel	19
Narayanan, Shridhar	Foundation for Neglected Disease Research, Bangalore, India	67
Nicklas, Danielle	Johns Hopkins University School of Medicine, Baltimore, USA	58

Presenter	Affiliation	Poster #
Ochsner, Urs	Crestone, Inc., Boulder, USA	73
Palme, Paul Robin	Martin-Luther-Universität Halle-Wittenberg (MLU), Halle (Saale), Germany	60
Palčeková, Zuzana	Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, USA	52
Panraksa, Yosita	Colorado State University, Fort Collins, USA	11
Park, Jiyun	Department of Microbiology, Graduate School of Medical science, Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea, Republic of	76
Phelps, Gregory	St. Jude Children's Research Hospital, Memphis, USA	79
Philley, Julie V.	Department of Medicine, University of Texas Health Science Center, Tyler, TX, USA	65
Pienaar, Elsje	Purdue University, West Lafayette, USA	56
Robinson, Richard	The Ohio State University, Columbus, USA	15
Ross, Brittany	Center for Microbial Dynamics and Infection at Georgia Tech, Atlanta, USA. Georgia Institute of Technology, Atlanta, USA	35
Santangelo, Maria de la Paz	IABIMO, Buenos Aires, Argentina	17
Sarathy, Jickky Palmae	Center for Discovery and Innovation - Hackensack Meridian Health, Nutley, USA	84
Schmalstig, Alan	University of North Carolina at Chapel Hill, Chapel Hill, USA	39
Shaw, Timothy	Queen's University Belfast, Belfast, United Kingdom	85
Shell, Scarlet	Worcester Polytechnic Institute, Worcester, USA	36
Singh, Sanjay	Department of Pulmonary Immunology, University of Texas Health Science Center, Tyler, Texas, USA	8
Sullivan, Jaryd	Dept of Microbiology & Immunology, McGill University, Montreal, Canada	69

Presenter	Affiliation	Poster #
Touré, Hamadoun	Université Paris-Saclay, UVSQ, INSERM, Infection et Inflammation, Montigny-Le-Bretonneux, France	38
Treen, Ryan	Wadsworth Center, Albany, USA	20
Vang, Charmie K.	National Jewish Health, Denver, USA	50
Weathered, Catherine	Purdue University, West Lafayette, USA	41
Whittel, Nicholas	Colorado State University, Fort Collins, USA	10
Wilt, Laura	St. Jude Children's Research Hospital, Memphis, USA	80
Wolf, Ian	Dept. of Immunology and Infectious Disease, Harvard T.H. Chan School of Public Health, Boston, USA	31

Short talks selected from abstracts

Wednesday June 1, 2022

Session # 2: Diagnostics and Biomarkers

14

Mycobacterium abscessus killing profiles reveal clinical outcomes independent of drug resistance

Alexander Jovanovic[1], Frederick Bright[1], Basil Wicki[1], Loïc Sauteur[1], Andreas Wüst[1], Dorothy M Grogono[2], Michael Tamm[3], Philippe Dehio[1], Johannes Nemeth[4], Michael Abanto[1], Rachel Thomson[5], Scott Bell[6], Andres R Floto[7], Lucas Boeck[1]

[1]*Department of Biomedicine, University of Basel, Basel, Switzerland.* [2]*Cambridge Centre for Lung Infection, Royal Papworth Hospital, Cambridge, United Kingdom.* [3]*Clinic of Pulmonary Medicine, University Hospital Basel, Basel, Switzerland.* [4]*Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, Zürich, Switzerland.* [5]*Gallipoli Medical Research Institute, The University of Queensland, Brisbane, Australia.* [6]*Children's Health Research Institute, The University of Queensland, Brisbane, Australia.* [7]*Molecular Immunity Unit, University of Cambridge Department of Medicine, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Abstract

Antimicrobial susceptibility testing (AST) is critical in basic biology, drug discovery and treatment guidance of infectious diseases. However, in chronic lung infections, AST often correlates poorly with treatment success. AST evaluates growth across antibiotic concentrations, but not bacterial killing (drug tolerance), which may be required to clear long-lasting infections. By overcoming the poor scalability and reproducibility of colony-forming units, we evaluate drug tolerance in Mycobacterium abscessus, a particularly difficult-to-treat pathogen causing increasing rates of chronic pulmonary infections globally.

To investigate drug tolerance at scale, we developed an experimental and analytical high-content live-cell imaging platform that allowed testing thousands of conditions. In brief, we immobilised, successively imaged, and then tracked around 50 Mio individual bacteria over 96 hours to evaluate single-cell dynamics and population time-kill kinetics. Using our approach, we analysed drug tolerance of 11 drugs, at three concentrations, across 142 M. abscessus isolates from different Cystic Fibrosis patients. We observed various time-kill profiles across and within drugs tested, indicating that bacterial killing is a fundamental bacterial phenotype. We demonstrate that drug tolerance is heritable and associated with clinical outcomes independent of AST. Finally, we reveal several bacterial mechanisms potentially underlying mycobacterial killing, using genome-wide association studies. Our findings highlight the relevance of drug tolerance in mycobacterial infections, potentially improving clinical guidance, and enforcing sterilising drug development.

15

Immune biogeography of nontuberculous mycobacteria (NTM) infected airways in people with cystic fibrosis (PwCF)

Don Hayes Jr.[1], *Rajni Kant Shukla*[2], *Yizi Cheng*[1], *Emrah Gecili*[1], *Rhonda Szczesniak*[1], *Assem Ziady*[1], *Jason Woods*[1], *Luanne Hall-Stoodley*[2], *Namal Liyanage*[2], *Richard Robinson*[2]

[1]*Cincinnati Children's Hospital Medical Center, Cincinnati, USA.* [2]*The Ohio State University, Columbus, USA*

Abstract

Nontuberculous mycobacteria (NTM) are an increasingly common cause of respiratory infection in people with cystic fibrosis (PwCF). Relative to those with no history of NTM infection (CF-NTM^{NEG}), PwCF and a history of NTM infection (CF-NTM^{POS}) are more likely to develop severe lung disease and experience complications over the course of treatment. In other mycobacterial infections (e.g. tuberculosis), an overexuberant immune response causes pathology and compromises organ function; however, since the immune profiles of CF-NTM^{POS} and CF-NTM^{NEG} airways are largely unexplored, it is unknown which if any immune responses distinguish these cohorts or concentrate in damaged tissues. Here we evaluated lung lobe-specific immune profiles of three adult cohorts (CF-NTM^{POS}, CF-NTM^{NEG}, and non-CF controls) and found that CF-NTM^{POS} airways are distinguished by a hyper-inflammatory cytokine profile. Importantly, the CF-NTM^{POS} airway immune profile was dominated by B cells, classical macrophages and the cytokines which support their accumulation. These and other immunological differences between cohorts, including the near absence of NK cells and complement pathway members, were enriched in the most damaged lung lobes. The implications of these findings for our understanding of lung disease in PwCF are discussed, as are how they may inform the development of host-directed therapies to improve NTM disease treatment.

Session # 3: Genetics and Physiology of NTM

33

High-density transposon mutagenesis in Mycobacterium abscessus identifies an essential penicillin-binding lipo-protein (PBP-lipo) involved in septal peptidoglycan synthesis and antibiotic sensitivity

Chidiebere Akusobi[1,2], *Bouchra S. Benghomari*[3], *Ian D. Wolf*[2], *Junhao Zhu*[2], *Shreya Singhvi*[2], *Charles L. Dulberger*[2], *Thomas R. Ioerger*[4], *Eric J. Rubin*[2]

[1]*Harvard Medical School, Boston, USA.* [2]*Harvard T.H. Chan School of Public Health, Boston, USA.* [3]*Northeastern University, Boston, USA.* [4]*Texas A&M University, College Station, USA*

Abstract

Mycobacterium abscessus (Mab) is a rapidly growing non-tuberculous mycobacterium that causes a wide range of infections. Treatment of Mab infections is difficult because the bacterium is intrinsically resistant to many classes of antibiotics. Developing new and effective treatments against Mab requires a better understanding of the unique vulnerabilities that can be targeted for future drug development. To achieve this, we identified essential genes in Mab by conducting transposon-sequencing (TnSeq) on the reference strain, ATCC 19977. We generated ~51,000 unique mutants and used this high-density library to identify 362 genes essential for in vitro growth. To investigate species-specific vulnerabilities, we further characterized MAB_3167c, a predicted penicillin-binding-lipoprotein (PBP-lipo) that is essential in Mab and non-essential in M. tuberculosis (Mtb). We found that PBP-lipo primarily localizes to the septum as cells grow and prepare to divide. Knockdown of PBP-lipo causes cells to elongate, develop ectopic branches, and form multiple septa. Dual knockdown of PBP-lipo with PBPs, PbpB, DacB1, and a carboxypeptidase, MAB_0519 lead to synergistic growth arrest. In contrast, these genetic interactions were absent in the Mtb model organism, M. smegmatis, indicating that the PBP-lipo homologs between the two species exist in distinct genetic networks. Finally, repressing PBP-lipo sensitized the reference strain and 11 Mab clinical isolates to several classes of antibiotics, including the beta-lactams, ampicillin and amoxicillin by greater than 128-fold. Altogether, this study presents PBP-lipo as a key enzyme to study Mab specific processes in cell wall synthesis and importantly positions PBP-lipo as an attractive drug target to treat Mab infections.

34

Lineage analysis of Mycobacterium abscessus subsp. abscessus isolates from a treatment refractory infection reveals genomic adaptations over a five-year period

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Abstract

A longitudinal series of Mycobacterium abscessus (MAB) isolates was recovered from a young man with advanced cystic fibrosis (CF) related lung disease who underwent over three years of unsuccessful antibiotic treatment for a MAB lung infection. This infection was ultimately resolved with the addition of bacteriophage therapy. Genomic analysis spanned the first positive culture through culture conversion, enabling an in-depth evaluation of population dynamics and adaptations to treatment. Methods: A total of 40 isolates, collected over 1,800 days underwent whole genome sequencing. The isolate population was analyzed for single nucleotide polymorphisms, time-scaled phylogeny, mutation rate, and accessory

genome composition. Results: Isolates recovered prior to antibiotic treatment were primarily clonal within the dominant circulating clone (DCC1) of MAB. With the initiation of antibiotic therapy, a population shift occurred with the emergence of adapted sublineages and evidence of parallel evolution of functional mutations across clades. With the initiation of phage therapy, the MAB population became genetically less diverse, and isolates of only one sublineage were recovered prior to sputum culture conversion to negative. Together, the isolate population demonstrated a mean substitution rate of 2.7×10^{-7} mutations/site/year, and the common ancestor was dated approximately 30 months prior to recovery of the first isolate. Pan genome analysis revealed a substantial reduction in accessory genes after antibiotic and phage treatment, consistent with selection for a persistent and homogeneous population. Conclusions: This unprecedented view of MAB airway infection simultaneously reveals the genetic stability of a MAB population and adaptive mutations resulting from sustained selection pressures.

35

Identification of molecular determinants that govern morphotype-specific physiology in Mycobacteroides abscessus

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Abstract

Little is known about the mechanisms underlying the physiological and behavioral differences between Mycobacteroides abscessus' morphotypes, smooth and rough. Although genetically similar, the morphotypes differ in antimicrobial tolerance, multicellular structures, immune activation, and patient disease progression. Transition from smooth to rough is due to loss of the hydrophilic surface-exposed glycopeptidolipid layer. It is unclear if this is the only driving force leading to phenotypic divergence. To investigate if the morphotypes possess other molecular mechanisms governing phenotypic differences, we generated transposon sequencing libraries in a ATCC19977 smooth and rough isolate. Although many essential genes are shared (441) under a nutrient-rich condition, smooth and rough MAB morphotypes have 165, and 59 unique essential genes, respectively. Further utilizing the Tn-seq libraries, we asked if the morphotypes utilize distinct genes for fitness during a skin abscess infection. Notably, the two morphotypes displayed more divergent essential genes compared to in vitro conditions with smooth requiring 138 unique genes, rough 109, and both requiring 137 genes not essential in vitro. These results indicate smooth elicits stress-induced DNA repair for in vivo fitness, while the rough requires several membrane proteins. By subjecting the libraries to several host-mimicking cues, we aim to deconvolve which and how each morphotype responds to specific cues during infection. Together, our findings show that smooth and rough morphotypes contain distinct genetic determinants underlying MAB fitness during infection and shed light on how the morphotypes differentially interact with the host.

36

The sRNA B11 controls virulence-associated phenotypes in Mycobacteroides abscessus

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Abstract

Little is known about the roles of small regulatory RNAs (sRNA) in Mycobacteroides abscessus. We found that deletion of the sRNA B11 in a smooth strain caused an intermediate smooth/rough morphology, increased antibiotic resistance, increased virulence in infection models, stronger innate immune activation, and increased transport to lysosomes. We identified several clinical isolates with B11 mutations. We used RNAseq to investigate the effects of B11 on gene expression and test the impact of two clinical mutations. ~230 genes were differentially expressed in ΔB11 compared to a complemented strain. Most of the genes differentially expressed in ΔB11 showed similar expression trends in strains with the clinical mutations, suggesting the clinical mutations caused partial loss-of-function. B11 has two C-rich loops previously found to repress expression in M. smegmatis by base-pairing to complementary sequences in ribosome binding sites (RBSs) of mRNAs. Among the genes upregulated in the ΔB11 mutant, there was a strong enrichment for the presence of B11-complementary RBSs. Comparing the proteomes of WT and ΔB11 strains likewise revealed a strong enrichment for B11-complementary RBSs in genes encoding upregulated proteins. Genes upregulated in ΔB11 included components of the virulence-associated ESX-4 secretion system. One of these had a B11-complementary RBS and fusing the RBS to a reporter made the reporter suppressible by B11. Together, our data show that B11 is a negative regulator with pleiotropic effects on gene expression and clinically important phenotypes in M. abscessus. To our knowledge, this is the first report of the role of an M. abscessus sRNA.

Session # 4: Pathogenic Strategies of NTM and Host Immune Response to NTM Infections

54

Unravelling the pathogenicity of Mycobacterium abscessus clinical isolates in CF pulmonary epithelial cell and mouse models of respiratory infection

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Abstract

Mycobacterium abscessus (MA) infections in Cystic fibrosis (CF) patients display heterogeneous clinical outcomes. To date, the contribution to MA pulmonary disease (MA-PD) development by dominant circulating clones (DCCs) or morphotypes, remains to be elucidated. We aim at defining pathogenicity of CF MA clinical strains in CF pulmonary epithelial cell and mouse models of MA respiratory infection.

We collected eleven longitudinal MA strains isolated from five patients both at the early asymptomatic and MA-PD phase. We performed morphotype (rough and smooth phenotype) and whole genome sequencing (WGS) analysis. Moreover, we studied the host response induced by CF isolates in CF epithelial cells (CFF-16HBEgeCFTRΔF508) by host RNA sequencing and cytokines release. We also tested the virulence of MA clinical strains in mouse models of lung infection.

Epithelial cells infected with DCC1 strains displays a higher pro-inflammatory response than DCC2 strains. Moreover, we found out that morphotype (smooth vs rough strains) is the main bacterial feature driving over 3000 host differentially expressed genes. This was confirmed also by the evaluation of IL6 and IL8 protein levels upon infection. Then, we tested the in vivo pathogenicity of two longitudinal strains from the same patient, belonging to DCC1 and displaying a different morphotype. Longitudinal persisted rough strain displayed a higher bacterial burden and pro-inflammatory response, such as airway monocyte recruitment, than smooth strain in acute and chronic mouse models of lung infection.

Our findings suggest that rough DCC1 strains persisted within CF lung may cause more severe respiratory infections.

Supported by Italian_CF_foundation FFC#23_2020

55

Defining complex mechanistic interactions and responses by macrophages during Mycobacterium abscessus infection

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Abstract

Mycobacterium abscessus (MAB) is a highly antibiotic resistant, rapidly growing non-tuberculous mycobacterium that infects patients with chronic lung diseases, like cystic fibrosis. Phagocytosis by macrophages and subsequent release of cytokines is critical to neutralize and terminate invading

pathogens. While interactions between MAB and macrophages contribute to its pathogenesis, how macrophages bind, phagocytose, and ultimately neutralize MAB during infection remains largely unknown. These interactions are further complicated by the ability of MAB to transition from a smooth to rough morphology during infection. We hypothesize that understanding key MAB-macrophage interactions will identify critical host targets that can be leveraged to prevent infection. To test this hypothesis, we developed new fluorescent reporters in smooth and rough MAB and optimized a range of macrophage assays to elucidate MAB-macrophage interactions both with and without antibiotic treatment. We used these tools to conduct a forward genetic screen using a genome-wide CRISPR-Cas9 knockout library in immortalized bone marrow derived macrophages to identify host pathways that contribute to MAB uptake four hours following infection. Our results show glycosaminoglycan (sGAG) synthesis in macrophages is required to efficiently take up smooth MAB during early infection. We are now testing how key regulators of sGAG biosynthesis, UGDH, B3GLCT, B4GALT7 and B3GAT3, influence macrophage interactions with smooth and rough MAB. Future work will determine how sGAG pathways modulate rough MAB uptake in both bone marrow-derived macrophages and a novel alveolar-like macrophage model with the goal of defining key mechanisms driving host-pathogen interactions in the lungs.

56

An Agent-Based Model to Assess the Contribution of Menopause-Associated Immunological Changes to Increased Risk of MAC Infection

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Abstract

The mechanistic reasons for the disproportionate number of Mycobacterium avium Complex (MAC) cases in post-menopausal women, compared to pre-menopausal, remain unclear. We have developed an agent-based model of the early interactions between bacteria and host immune cells in the lung airway to explore infection progression at both the intracellular and tissue scales. We generated simulations that represent pre- and post-menopausal patients by scaling model parameters for innate immune processes known to be disrupted post-menopause: macrophage recruitment, phagocytosis, and bacterial killing. Using this model, we can quantify risk of each of these individually or interplay among these.

In our post-menopausal simulations, we find a significant increase in bacterial loads across all three phenotypes (sessile, planktonic, and intracellular) and infected macrophages, and a decrease in healthy macrophages. This finding is contrasted by a decrease in total phagocytosis. Slowed macrophage phagocytosis leads to increases in extracellular bacteria. While decreased killing rates lead to increased accumulation of intracellular bacteria.

Taken together, these results suggest that menopause-associated parameters drive an overall increase in all bacterial phenotypes and an increase in the proportion of macrophages that are infected. The

increase in infected macrophages post-menopause suggest that the accumulation of extracellular bacteria is enough to overcome impaired phagocytosis, while the higher intracellular bacteria levels indicate that bacterial killing is the limiting factor in post-menopausal patients' bacterial elimination. Thus, menopause could affect the balance between healthy and infected macrophages which, in turn, will have significant impacts as T-cells begin to respond.

57

Immunogenicity and protection against Mycobacterium avium with a heterologous RNA prime and protein boost vaccine regimen

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Abstract

Pulmonary lung disease caused by nontuberculous mycobacteria (NTM) is becoming an increasing health threat. Toxic side-effects from anti-mycobacterial drugs can affect patient compliance, preventing the completion of drug treatment. A vaccine against pathogenic NTM, used as an adjunct to drug treatment, could reduce treatment time and lower the risks of severe side-effects.

Prophylactic efficacy of two delivery platforms for vaccination against Mycobacterium avium (M. avium) were tested. The vaccine antigen, ID91, includes 4 mycobacterial antigens: Rv3619, Rv2389, Rv3478, and Rv1886. ID91+GLA-SE is effective against a clinical NTM isolate, M. avium 2-151smt. Here we extend these results and show that a heterologous prime/boost strategy with repRNA-ID91 (replicating RNA) followed by protein ID91+GLA-SE boost is superior to the subunit-protein given as a homologous prime/boost regimen. The repRNA-ID91/ID91+GLA-SE regimen elicited CD4+ Th1 immune responses to four ID91 component proteins whereas the homologous protein prime/boost regimen induced responses to Rv1886 and Rv319 only. RepRNA-ID91/ID91+GLA-SE also induced TNF-secreting CD8+ T cells.

Finally, both vaccine regimens elicited protection against M. avium 2-151smt in Beige mice, measured by a significant decrease in bacterial load in the lung and spleen. Future studies are needed to determine whether these regimens induce long-lived memory immune responses or are protective against other NTM. Furthermore, we wish to test these vaccine regimens in the context of therapy as an adjunct to drug treatment.

Research was supported by the NIH under R21AI142267-01A1 and is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Session # 5: Intervention Strategies and Clinical Trials

83

Understanding antibiotic penetration into mycobacterial biofilms using NanoSIMS

Winifred Akwani[1,2], *Ian Gilmore*[2], *Paulina Rakowska*[3], *Mark Chambers*[1], *Greg McMahon*[2], *Suzie Hingley-Wilson*[1]

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Abstract

Mycobacterium abscessus is an opportunistic, drug-resistant, nontuberculous mycobacteria (NTM) pathogen associated with chronic pulmonary infections, especially in individuals with cystic fibrosis. Biofilm formation can take place along the alveolar walls of such patients following inhalation of **M. abscessus** from environmental reservoirs. These biofilms have an increased level of antimicrobial resistance (AMR) and are difficult to eradicate. Treatment for **M. abscessus** infections often requires administration of a cocktail of antibiotics for over two years and is frequently unsuccessful. Bedaquiline (BDQ), is an approved antibiotic used for the treatment of multidrug- resistant tuberculosis, which inhibits mycobacterial ATP synthase and evidence has shown **in vitro** efficacy against NTMs. The question being addressed is whether the increased AMR and treatment time in **M. abscessus** infection is due to lack of antibiotic penetration into the biofilm**.** The susceptibility of **M. abscessus** grown as planktonic bacilli and biofilms to the antibiotic bedaquiline (BDQ) was measured as the minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC), respectively. The MBEC of BDQ was 16 times higher (4µg/ml) compared with the MIC (0.25µg/ml). In addition, nano scale secondary ion mass spectrometry (NanoSIMS) was used to assess the penetration of BDQ into **M. abscessus** within biofilms. This was achieved by analysing the ratio of the uptake of Br^- ion in BDQ with the organic elements in the individual cells of **M. abscessus** biofilms. Understanding antibiotic penetration and AMR generation in NTM biofilms could lead to the development of novel treatment strategies.

84

Mycobacterium tuberculosis DprE1 inhibitor OPC-167832 is active against Mycobacterium abscessus

Jickky Palmae Sarathy, *Matthew D. Zimmerman, Véronique Dartois, Martin Gengenbacher, Thomas Dick*

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Abstract

Inhibitors of Mycobacterium tuberculosis decaprenylphosphoryl-β-D-ribose oxidase (DprE1) have emerged as promising candidates for tuberculosis (TB) treatment. A few DprE1 inhibitors have been tested against Mycobacterium abscessus (Mab) and found to be inactive, thus calling into question the 'vulnerability' of the Mab DprE1 homolog (Mab_0192c). As part of our strategy to identify new anti-Mab drugs by screening TB actives, we screened current DprE1 inhibitors and confirmed that most of the compounds lacked anti-Mab activity. Surprisingly, we found the carbostyril derivative OPC-167832 to be active. This clinical drug candidate displayed potent activity against subspecies reference strains and a collection of clinical isolates of Mab. Drug potency interaction studies showed that the compound did not antagonize the activity of commonly used Mab antibiotics, suggesting that OPC-167832 could be co-administered with current drugs. Importantly, OPC-167832 showed efficacy in a Mab mouse infection model. Isolation and sequencing of spontaneous resistant mutants confirmed the Mab DprE1 homolog as the compound's target. This sequencing exercise also revealed mutations in the homolog of RNA polymerase sigma factor SigA (Mab_3009) as a major resistance mechanism in mutants without Mab DprE1 mutations. It is noteworthy that we observed a dramatic potency difference in Middlebrook 7H9 vs cation-adjusted Mueller Hinton (CAMH) broth, which was uncovered to be due to protein binding in the culture medium. In conclusion, we validated the DprE1 homolog as an attractive drug target for Mab and identified OPC-167832 as a novel, oral and bactericidal drug candidate for the treatment of Mab lung disease.

85

Human mesenchymal stromal cells inhibit Mycobacterium avium growth in in vitro and in vivo models of pulmonary infection

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Abstract

Aims

New therapeutic strategies are needed for Mycobacterium avium pulmonary infection. Mesenchymal stromal cells (MSCs) are mature multipotent cells with antimicrobial and immunomodulatory properties. We investigated the therapeutic potential of MSCs using in vitro and in vivo models of M. avium pulmonary infection.

Methods

Human monocyte-derived macrophages (MDMs) were infected with M. avium Chester strain and treated with human bone marrow-derived MSCs. Colony-forming units (CFU) were counted for extracellular and intracellular bacteria after 72 hours.

Balb/c mice were administered aerosolised M. avium and treated with $1x10^6$ intravenous human bone marrow-derived MSCs (or placebo) at 21 days and 28 days post infection (p.i.). Lungs, liver and spleen were harvested at day 42 p.i. for bacterial counts. Cytokines were quantified by ELISA.

Results

MSCs reduced numbers of intracellular bacteria in MDMs over 72 hours (median 40% reduction, IQR 20-50%, $p<0.05$). Extracellular CFU were unaffected by MSCs. MSC treatment of infected MDMs led to increased concentrations PGE2 (median 10.1-fold, $p<0.05$). Blocking MSC PGE2 production by COX2 inhibition led to abrogation of their antimycobacterial effect, which was restored through adding exogenous PGE2.

MSC-treated mice had a median 20% reduction in pulmonary CFU (IQR 10-40%, $p<0.05$). but no change in liver and splenic bacterial counts.

Conclusions

Human MSCs inhibited intracellular survival of M. avium in macrophages and reduced pulmonary counts in a mouse model of chronic NTM infection. Cellular studies suggest MSCs disrupt intracellular M. avium growth in a COX2/PGE2-dependent manner. Further evaluation of MSCs as an adjunctive therapy in anti-mycobacterial treatment is warranted.

86

Shredding Intracellular NTMs with a Macrophage-Targeted Enzymatic Cocktail

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Abstract

To address intracellular mycobacterial infections, Endolytix developed a cocktail of four enzymes that catalytically attack 3 layers of the mycobacterial envelope that is delivered to macrophages through a targeted drug delivery vehicle. This unique combination of enzymes leverages some enzymes encoded by bacteriophages while others come from other organisms thus allowing degradation of the mycobacterial envelope from the outside in, reversing traditional bacteriophage-based approaches while avoiding bacterial surveillance pathways that cleave bacteriophage genomes. Lysin A, a mycobacteriophage encoded protein is thought to cleave the peptidoglycan layer. Lysin B, also mycobacteriophage derived, is an esterase that hydrolyzes the linkage between arabinogalactan and mycolic acid layers. The problem of providing access to the substrates of Lysin A and Lysin B exogenously was addressed by adding enzymes that would degrade the extracellular capsule shown to present in M. tuberculosis and expected to be prevalent in NTMs as determined by host range studies we have conducted so far. We demonstrate our drug formulation is bactericidal in both in vitro and ex vivo experiments. Here we demonstrate a mechanism of action that results in rapid mycobacterial cell death by fragmentation into subcellular fragments. We demonstrate the ability to rescue macrophages from the necrotic cell death of mycobacterial infection.

87

The arylvinylpiperazine amide AX-35 targets the cytochrome bc_1 in Mycobacterium abscessus.

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Abstract

In cystic fibrosis patients, non-tuberculous mycobacteria (NTM) are opportunistic, intracellular pulmonary pathogens that contribute to lung function deterioration and, ultimately, morbidity due to respiratory failure. Of the NTM respiratory pathogens, Mycobacterium abscessus complex (MABSC) poses additional treatment complications as they are notoriously drug-resistant. Despite belonging to the same genus as Mycobacterium tuberculosis (M.tb), MABSC is resistant to most antitubercular drugs, resulting in only a handful of active drugs against these bacteria. As part of our routine screening against other mycobacterial species, one of the lead anti-TB candidates, the cytochrome bc_1 inhibitor AX-35, was found to be active against MABSC isolates in vitro (MIC$\leq$ 15µg/mL) and ex vivo (M. abscessus-infected macrophages). Isolation and characterization of AX-35 resistant mutants (MIC AX-35 $\geq$ 250µg/mL) revealed the presence of a chimeric gene resulting from the recombination of MAB_2467 in MAB_1966c. In M. abscessus, both genes encode for the b subunit of the cytochrome bc_1 oxidase (QcrB). Further analysis, comprising gene inactivation and ATP-depletion assay, suggested that MAB_1966c is the target of AX-35. Interestingly, transcriptomic analysis of M. abscessus treated with AX-35 revealed that the compound triggered the expression of MAB_2467 and the alternate terminal oxidase, the cytochrome bd. In M.tb, in the absence of the cytochrome bd oxidase, QcrB inhibitors, including AX-35, are bactericidal. However, in M. abscessus, AX-35 had bacteriostatic activity. These results suggested that, in the presence of AX-35, MAB_2467 may compensate for the absence of the cytochrome bd oxidase.

Poster Presentations

Poster Session 1 - Wednesday June 1

3:30- 6:00 pm

Epidemiology and Clinical Aspects of NTM Diseases

1

GLYCOPEPTIDOLIPID (GPL) DEFECTS DO NOT AFFECT SURVIVAL OF ROUGH MYCOBACTERIUM ABSCESSUS ISOLATES ON FOMITES AND IN CHLORHEXIDINE, AS COMPARED TO THEIR ISOGENIC SMOOTH COUNTERPARTS

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Abstract

BACKGROUND: Human transmission of Mycobacterium abscessus, and its extent among patients has been an issue of continued debate in the past several years. Within-patient host-driven mutations in M. abscessus, and specifically Glycopeptidolipid-defected Rough isolates were suggested to increase bacterial virulence while reducing transmissibility on fomites.

METHODS: We used transposon technology to create fully isogenic Rough (GPL-defective) (Tn_4099c) and compare it to the isogenic parent strain (ATCC 19977). Survival on fomites was determined by spotting the isolates and retrieving them at designated time points. This was repeated as a competition experiment using a mixture of fluorescent M. abscessus 19977 (Smooth, red fluorescence) and the Tn_4099c mutant (Rough, green fluorescence). Survival ability in chlorhexidine solution (Septal Scrub® Teva) was performed using a disinfectant killing-assay for mycobacteria.

RESULTS: Despite significant bacterial killing in all assays, we found no survival advantage to either GPL-defected Rough or Smooth morphotype – both on fomites and in chlorhexidine.

CONCLUSIONS: Our findings suggest that while transmission fitness may be altered due to some within-host evolutionary changes, decreased transmissibility of clinical strains cannot be attributed to the GPL-synthesis defect alone. Our results continue to encourage common infection control practices, including appropriate drying and disinfection of respiratory equipment to minimize the possibility of patient-to-patient transmission.

2

Risk factors for Non-Tuberculous Mycobacteria Infections in Solid Organ Transplant recipients: a multinational case-control study

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Abstract

Background: Risk factors for nontuberculous mycobacteria (NTM) infections after solid organ transplant (SOT) are poorly characterized. We aimed to describe clinical characteristics and identify risk factors for NTM infections after SOT.

Methods: Retrospective, international, 1:2 matched case-control study that included SOT recipients ≥12 years old with NTM infection from January 1st, 2008, to December 31st 2018. Controls were matched on transplanted organ, center, and post-transplant survival equal or greater than the time to NTM diagnosis. Demographics, comorbidities, predisposing factors, immunosuppressive medication usage, and NTM disease characteristics were collected. Univariable and multivariable conditional logistic regression on matched pairs were used to identify NTM risk factors.

Results: Analyses included 85 cases and 169 matched controls; 59% were male, 88% white and median (IQR) age at time of transplant was 54 years (40-62). NTM infection was found in kidney (42%), lung (35%), heart and liver (11% each), and pancreas transplant recipients (1%), median time from transplant to infection was 21.6 months (5.3-55.2), cases were older (61 vs 55 years), more frequently on systemic

steroids (85% vs 75%) and had a lower median lymphocyte count (0.8 vs 1.2) (all $P<0.05$). In the multivariable model, older age at time of transplant (aOR 1.04; 95%CI, 1.01-1.07), prior hospital admission (3.14; 1.41-6.98), receipt of antifungals (5.35; 1.7-16.91), and of lymphocyte-specific antibodies (7.73, 1.07-56.14) were significantly associated with NTM infection.

Conclusion: Risk of NTM infection in SOT recipients was associated with older age at the time of transplant, prior hospital admission, receipt of antifungals and lymphocyte-specific antibodies.

3

Investigations of Healthcare-Associated Nontuberculous Mycobacteria Infections Associated with Contamination of Medical Products – United States, 2013-2022

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Abstract

Background:

Nontuberculous mycobacteria (NTM) are opportunistic pathogens found in soil and water and can exploit a myriad of pathways to infect patients in healthcare settings. We describe Centers for Disease Control and Prevention (CDC) consultations for healthcare-associated NTM infections and outbreaks associated with intrinsic (during product manufacturing) and extrinsic (at the point of use) contamination of medical products.

Methods:

CDC maintains records of consultations with state or local health departments related to healthcare-associated outbreaks and infection control breaches. We reviewed consultations involving NTM species as the primary pathogen of concern, with product contamination documented as an infection control area of concern (excluding heater cooler device consultations), from January 1, 2013 through March 1, 2022.

Results:

We identified nine consultations involving a range of 1-101 patients (median=6). Species identified included M. abscessus in 3 consultations , M. chelonae (3), M. porcinum (1), M. fortuitum (1), and a novel NTM species (1). Settings included 4 outpatient clinics (pediatric, oncology, podiatry, and ophthalmic surgery), 2 hospitals, 1 worksite vaccine clinic, 1 med spa, and 1 home. There was concern for extrinsic contamination in seven consultations (two involving vaccines) and for both intrinsic and extrinsic contamination in two consultations. Other contaminated products included total parenteral nutrition, injectable steroids, saline flushes, beta-human chorionic gonadotropin, intrathecal hydromorphone, injectable artichoke fat, and a humidifier used during ophthalmic surgery. Injection safety breaches were described in six consultations.

Conclusions:

Our experiences consulting on healthcare-associated NTM revealed issues related to medical product contamination, particularly injectable medications due to facility-level contamination.

4

Link between disease incidence, access to drinking water and M. ulcerans genetic diversity

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Abstract

Buruli ulcer, an emerging neglected tropical disease, is caused by Mycobacterium ulcerans, mainly in poor areas of West and Central Africa. The development of appropriate preventive strategies is hampered by an incomplete understanding of the epidemiology and transmission of the disease. We investigated the impact of the drilling of wells on Buruli ulcer incidence. We found a strong inverse correlation between the incidence of Buruli ulcer and the number of new wells drilled. We also identified well-drilling as a protective factor for the population in a case-control study. This study is the first report demonstrating the positive impact of well drilling installation in the field of Neglected Tropical Diseases, with possible implications also in such other fields. In a second study conducted in the same area, we carried-out a local-scale spatial clustering model and deciphered the genetic diversity of the bacteria. Using 179 M. ulcerans strains, we conducted a phylogeographic analysis combining whole genome sequencing with spatial scan statistics. The eight distinct genotypes identified were found by no means randomly spread over the studied area. They were divided into three different geographical clusters, associated with landscape characteristics highlighting the feature of the bacteria to evolve independently and differentially depending on their localization in a specific ecological reservoir. Our integrated approach, combined field studies, epidemiology and genomic analysis provided a method and a tool allowing the identification of delimited high-risk contamination areas.

5

Interpreting susceptibility results for clinical use of novel drugs for rapidly growing mycobacteria

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Abstract

Introduction

CLSI susceptibility breakpoints are not available for several recently approved antibiotics being used for treatment of nontuberculous mycobacteria (NTM) infections. We present minimum inhibitory concentrations (MIC) of rapidly growing mycobacteria (RGM) from our institution and suggested interpretation for their clinical use.

Methods

M. abscessus, M. chelonae, and M. fortuitum were identified by partial sequencing of the 16S rRNA gene and hsp65 gene, and Mass Spectrometry of protein profiles. MICs (µg/ml) were determined in CA-MHB by microdilution methodology as recommended by CLSI M24, 3rd ed.; breakpoints where available were as recommended by CLSI M62.

Results

From 2020-2021 we identified 22 RGM isolates from 20 patients- 12 pulmonary and 10 extra-pulmonary. Twelve isolates were M. abscessus, 6 M. fortuitum, 3 M. chelonae, and 1 M. smegmatis. The MIC50 (range) for 11 M. abscessus isolates was 0.12 (0.06-0.25) for bedaquiline, 0.5 (0.25-1) for clofazimine, 128 (2-128) for delafloxacin, 1 (0.12-4) for omadacycline, and 1 (0.12-128) for tedizolid. In comparison, the linezolid MIC50 was 16 (2.0->32.0), with 5 considered susceptible. None were susceptible to moxifloxacin (MIC >8 for all).

Discussion

Based on review of the literature and known pharmacokinetics of these drugs, M. abscessus and other RGM could be considered susceptible with potential role for inclusion in treatment regimens at the following MICs: bedaquiline ≤0.25, clofazimine ≤1, Omadacycline ≤1, tedizolid ≤2. More isolates would be considered susceptible to tedizolid compared to linezolid by this criteria. Fluoroquinolone MICs were too high for most M. abscessus isolates, though may be options for other RGMs.

6

Healthcare-Associated Links in Transmission of Nontuberculous Mycobacteria Among People with Cystic Fibrosis (HALT NTM): A Multi-Center Study

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Abstract

Background: Nontuberculous mycobacteria (NTM) pose an increasing concern to individuals with cystic fibrosis (CF). We developed an evidence-based, standardized approach to investigate healthcare-associated NTM transmission. Hypothesis: Clusters of highly similar strains of NTM in people cared for at the same CF Care Center may arise from patient-to-patient transmission and/or acquisition from healthcare environmental sources. Methods: Whole genome sequencing (WGS) of respiratory NTM isolates from CF Centers nationwide identified genetically similar clusters of NTM isolates. Epidemiological investigation, comparisons of respiratory and environmental isolates, and home residence watershed mapping was performed in 4 CF Care Centers. Results: In Center A, WGS analysis revealed 11 NTM clusters. Epidemiologic investigation revealed potential healthcare-associated transmission events in 2 (33%) M. abscessus clusters and 2 (100%) M. avium clusters. Respiratory and healthcare environmental isolate comparisons revealed no genetic similarity. One M. abscessus cluster, with no plausible healthcare-associated transmission, resided in the same watershed. In Center B, WGS analysis revealed 2 clusters (M. avium and M. chimaera), and both had potential opportunities for healthcare-associated transmission. Respiratory M. chimaera isolates revealed greater genetic similarity to a single hospital water biofilm isolate than to each other. Neither cluster had subjects residing in the same watershed. Two additional Center outbreak investigations are ongoing. Conclusions: Each healthcare-associated NTM investigation has unique findings. The presence of genetically similar isolates is insufficient to definitively demonstrate healthcare-associated NTM transmission. Use of a standardized epidemiological investigation, coupled with environmental sampling and watershed analysis, will improve our understanding of healthcare-associated NTM acquisition and transmission.

Diagnostics and Biomarkers

7

Antimicrobial Susceptibility Profile of Slowly Growing Mycobacteria in the United States

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Abstract

Antibiograms are cumulative summaries of susceptibility patterns but they are not widely available for slowly growing mycobacteria (SGM). Here we generate a comprehensive SGM antibiogram from the largest sample size to date.

Antimicrobial susceptibility test (AST) results for isolates received at the National Jewish Heath Mycobacteriology Laboratory between 01/01/2018 and 12/31/2020 were compiled. Only the first isolate from each patient was included. Species with <15 isolates were excluded. The M62 Clinical Laboratory Standards Institute (CLSI) guidelines were used to determine drug interpretations. Line probe assay (LPA) results for the rrs and rrl genes were also compiled for a subset of Mycobacterium avium complex (MAC) isolates.

AST results were evaluated for seven drugs across 4,822 SGM isolates. Within MAC, the susceptibility to amikacin had a range of 60 - 93%, and susceptibility to clarithromycin had a range of 94 – 100%. However, LPA results for MAC showed low frequencies of rrs mutations (≤1.2%) and rrl mutations (0.7 – 10.5%). Most non-MAC isolates had high rates of susceptibility to rifabutin, except for M. asiaticum (52%) and M. simiae (36%). On the other hand, non-MAC isolates had low rates of susceptibility to moxifloxacin except for M. haemophilum (100%), M. marinum (99%), and M. triplex (71%).

The antibiogram generated in this study showed substantial differences in susceptibility across MAC and other SGM species. Interestingly, the low frequency of detected rrs resistance mutations did not correlate with moderate phenotypic susceptibility to amikacin. This suggests the potential for other mutations or resistance mechanisms to amikacin in MAC.

8

Omadacycline and challenges with determining MIC of nontuberculous mycobacteria

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Abstract

Background: Lack of standardized methods to determine the of MIC is the primary reason that the laboratory results often does not correlates with the clinical response of the drugs used to treat pulmonary disease caused by nontuberculous mycobacteria (NTM).

Methods: We performed drug degradation studies and broth micro-dilution MIC experiments with Mycobacterium abscessus (Mab) ATCC#19977 and M. kansasii ATCC#12478 as well as 20 clinical isolates of each. Omadacycline was supplemented 50% daily to keep the concentrations constant as in the beginning of the experiment. The omadacycline concentrations ranged from 0.0625-128 mg/L.

Results: We found 66.48±19.38% loss in omadacycline potency every 24hr of incubation. For the rapidly growing NTM, Mab, the omadacycline MIC was recorded as 0.5 mg/L and 1 mg/L at the end of 24 hr and 48 hr of incubation, respectively, and the MIC50 and MIC90 of the Mab clinical isolates was recorded as 1 and 2 mg/L, respectively. For slowly growing NTM, Mkn, the MIC of ATCC#12478 strain after 7 days of incubation with no drug supplementation was recorded as 128 mg/L and 16 mg/L with 50% daily drug supplementation. The MIC50 and MIC90 for Mkn clinical isolates was 16 and 32 mg/L, respectively.

Conclusions: Conventional methods to determine omadacycline MIC for rapidly growing NTM may yield comparable results, however, for NTM that have slower doubling time (growth rate) compared to the rate of omadacycline degradation, alternative strategies are required, including daily drug supplementation.

9

Molecular evidence for Mycobacterium abscessus in NTM patient cohorts classified by sputum culture

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Abstract

Background: Mycobacterium abscessus (MAB) is an important CF pathogen. Sputum culture is the only test utilized for detection and assessment of treatment response, but it has low sensitivity due to required decontamination procedures. We hypothesized that a molecular assay could detect MAB DNA from total sputum DNA. Methods: A TaqMan real-time quantitative PCR (qPCR) assay was optimized for detection of MAB DNA and evaluated in 120 sputum samples from 86 subjects enrolled in the PREDICT and PATIENCE trials. MAB DNA abundance was extrapolated from an 8-point standard curve. Results: Of 120 samples, 24 (20%) detected MAB DNA by qPCR. When compared to culture results, the sensitivity and specificity of molecular MAB detection was 79% and 91%, with a positive predictive value of 61% and a negative predictive value of 96%. Among the 10 samples that were molecular MAB positive and culture negative, 5 were from subjects who had prior MAB positive cultures ranging from 62–1,402 days before sample collection, and 5 were from subjects with no history of positive MAB cultures. Among subjects with >1 sample, molecular detection in any sample had a sensitivity and specificity of 88% and 65% for a positive MAB culture within 30 days. Conclusions: Molecular amplification by qPCR using low inputs of total sputum DNA is sensitive and specific for MAB detection compared to airway culture. In some cases, qPCR may detect MAB that is nonculturable with current decontamination methods. Analysis of >1 sample per subject may improve the sensitivity of the assay.

10

Generation and Kinetics of Bioluminescent Reporter Strains of Non-tuberculosis Mycobacteria for Longitudinal In Vitro and In Vivo Study Design

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Abstract

Rapid-growing (RGM) and slow-growing (SGM) non-tuberculous mycobacteria (NTMs) cause medically significant and difficult to treat infections. As a result, there is an urgent need for improved therapeutics. A substantial hurdle in discovering new treatments is that in vitro activity and drug efficacy in animal models of infection do not correlate well with the clinical outcomes of NTM patients. This disconnect could result from in vitro and in vivo drug susceptibility testing relying on endpoint data collection and limited information about the in vivo bacterial response to the host environment and treatment exposure. A panel of ATCC standard and clinically derived NTM bioluminescent strains are being developed and characterized under defined in vitro growth conditions and animal models. The use of bioluminescence in drug assessment allows for the determination of in vitro and in vivo kinetics measured using the in vivo imaging systems (IVIS). Additionally, the bio-location and response to treatment can be imaged longitudinally after infection without animal sacrifice and organ homogenization, improving visualization and monitoring of tissue targeting during drug assessment. These growth models' development provides additional information to determine how bacteria respond to different treatments at progressive infection time points and across various tissues. This project will characterize the efficacy kinetics of in vitro and in vivo bacterial responses to combination and single drug therapies. Discerning bacterial responses under xenobiotic challenge within a host’s changing microenvironment will guide decisions in developing new TB regimens.

11

Early stage development of an automated microfluidic interface lateral flow immunoassay platform for lipoarabinomannan detection in urine

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Abstract

The enzyme-amplified lateral flow immunoassays (LFIAs) enhance sensitivity over traditional LFIA platform. However, this platform has tedious operation steps which adds to certain complexity for on-site analysis. In a point-of-care setting,in this work, a microfluidic interface enzyme-based LFIAs platform is reported for automated sample, buffer, and reagent addition resulting in time and step saving as well as amplifying signal intensity. The polyester film, double-sided adhesive tape, and nitrocellulose are the materials required for the device fabrication. All required reagents are spotted on the nitrocellulose membrane and the sample is used as the wash buffer to minimize steps. After sample loading, a colorimetric result can be detected within 15 min by naked-eye reading. We will present data on the development and optimization of the channel geometry to achieve a simple step enzyme amplified immunoassay. Lipoarabinomannan (LAM), a WHO identified urinary biomarker of active tuberculosis, is used to demonstrate the device feasibility and reliability. The initial device successfully detected LAM in phosphate buffer (PBS) as well as spiked urine samples. The limit of detection was achieved at 25 ng/mL of LAM.

12

Immunoglobulins in Saliva as a Culture-Independent Marker of Nontuberculous Mycobacterial Infection in Cystic Fibrosis

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Abstract

People with cystic fibrosis (pwCF) are at high risk for developing nontuberculous mycobacterial (NTM) lung infection and disease. Detection of these infections remains difficult due to the slow growth and low sensitivity of traditional sputum culture. Prior work has indicated utility in measuring anti-NTM immunoglobulins in plasma as a potential biomarker for NTM disease. Hypothesis: Anti-NTM IgA and IgG in saliva are a sensitive culture-independent biomarker for NTM disease. Levels of anti-NTM immunoglobulins in saliva were quantified by ELISA. Samples from subjects meeting ATS criteria for NTM lung disease within a year of their last culture with M. avium complex or M. abscessus (N=12) were compared to those negative for NTM (N=55). Patients with known NTM disease had a higher level of anti-NTM IgA and IgG compared to culture negative patients (p=0.0003 by t-test) with a sensitivity of 58% and specificity of 92% with a binary classification 2 SD above the mean. There was no difference in immunoglobulin levels between those with MAC or M. abscessus. A saliva biomarker has the potential to improve how we diagnose NTM disease in pwCF, particularly in children and adults unable to expectorate sputum. Although limited by sample size, our preliminary data from this study indicates that detection of high levels of anti-NTM IgA and IgG in the saliva of pwCF has the potential to be used as a culture-independent biomarker of NTM lung disease.

13

Prospective AnalysIs of uriNe LAM to Eliminate NTM Sputum Screening (PAINLESS) Trial

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Abstract

People with Cystic Fibrosis (pwCF) are at greatest risk for pulmonary NTM, however, a large majority of pwCF will not contract these infections. Annual sputum screening can be difficult in many with CF, and most CF Programs are not meeting this recommendation. Lipoarabinomannan (LAM), a cell wall lipoglycan of all mycobacteria species, is released into circulation from metabolically active or degrading bacteria and is found in the urine of infected CF patients albeit in low amounts (~3-10 ng/mL). Hypothesis: Urine LAM is a sensitive, non-invasive screening test to identify individuals with an extremely low risk of having a positive NTM sputum culture. Results: Starting in October 2020, pwCF (n=80) with no history of NTM infection have been enrolled in the PAINLESS trial, with annual urine LAM analysis and sputum NTM cultures obtained during clinical care. To date, a 100% correlation between a non-detectable urine LAM (when measured by Gas Chromatography/Mass Spectrometry) and a history of negative NTM sputum culture has been observed. Five participants have demonstrated positive urine LAM, and to date one has subsequently cultured positive for NTM by sputum. Conclusion: This trial is ongoing, and if successful, will demonstrate that periodic urine LAM screening may identify pwCF with negligible risk for a positive NTM culture, and eliminate the need for routine sputum screening in the absence of clinical suspicion. Potentially, urine LAM will also provide earlier detection than cultures in patients who are newly infected.

14

Mycobacterium abscessus killing profiles reveal clinical outcomes independent of drug resistance

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Abstract

Antimicrobial susceptibility testing (AST) is critical in basic biology, drug discovery and treatment guidance of infectious diseases. However, in chronic lung infections, AST often correlates poorly with treatment success. AST evaluates growth across antibiotic concentrations, but not bacterial killing (drug tolerance), which may be required to clear long-lasting infections. By overcoming the poor scalability and reproducibility of colony-forming units, we evaluate drug tolerance in Mycobacterium abscessus, a particularly difficult-to-treat pathogen causing increasing rates of chronic pulmonary infections globally.

To investigate drug tolerance at scale, we developed an experimental and analytical high-content live-cell imaging platform that allowed testing thousands of conditions. In brief, we immobilised, successively imaged, and then tracked around 50 Mio individual bacteria over 96 hours to evaluate single-cell dynamics and population time-kill kinetics. Using our approach, we analysed drug tolerance of 11 drugs, at three concentrations, across 142 M. abscessus isolates from different Cystic Fibrosis patients. We observed various time-kill profiles across and within drugs tested, indicating that bacterial killing is a fundamental bacterial phenotype. We demonstrate that drug tolerance is heritable and associated with clinical outcomes independent of AST. Finally, we reveal several bacterial mechanisms potentially underlying mycobacterial killing, using genome-wide association studies. Our findings highlight the relevance of drug tolerance in mycobacterial infections, potentially improving clinical guidance, and enforcing sterilising drug development.

15

Immune biogeography of nontuberculous mycobacteria (NTM) infected airways in people with cystic fibrosis (PwCF)

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Abstract

Nontuberculous mycobacteria (NTM) are an increasingly common cause of respiratory infection in people with cystic fibrosis (PwCF). Relative to those with no history of NTM infection (CF-NTM^{NEG}), PwCF and a history of NTM infection (CF-NTM^{POS}) are more likely to develop severe lung disease and experience complications over the course of treatment. In other mycobacterial infections (e.g. tuberculosis), an overexuberant immune response causes pathology and compromises organ function; however, since the immune profiles of CF-NTM^{POS} and CF-NTM^{NEG} airways are largely unexplored, it is unknown which if any immune responses distinguish these cohorts or concentrate in damaged tissues. Here we evaluated lung lobe-specific immune profiles of three adult cohorts (CF-NTM^{POS}, CF-NTM^{NEG}, and non-CF controls) and found that CF-NTM^{POS} airways are distinguished by a hyper-inflammatory cytokine profile. Importantly, the CF-NTM^{POS} airway immune profile was dominated by B cells, classical macrophages and the cytokines which support their accumulation. These and other immunological differences between cohorts, including the near absence of NK cells and complement pathway members, were enriched in the most damaged lung lobes. The implications of these findings for our understanding of lung disease in PwCF are discussed, as are how they may inform the development of host-directed therapies to improve NTM disease treatment.

Genetics and Physiology of NTM

16

A simplified method for Tn-mutant library creation and Tn-seq in Mycobacterium abscessus

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Abstract

Introduction: Transposon-mutant library creation is a powerful tool in microbiology research, but creation of a comprehensive Tn-library in M. abscessus using the currently available, kanamycin-selected mycoMar7 phage/transposon has proved difficult. Additionally, the transposon insertion-site sequencing is complicated and may appear intimidating to researchers less experienced in bioinformatics.

Methods: we constructed a novel transposition tool, using strict zeocin (rather than kanamycin) selection. This tool also allows a "modern" Tn-seq technique, based on MmeI restriction sites introduced in the Inverted Repeats of the transposon. Additionally, we developed a short, easy and efficient protocol for Tn-seq, allowing better identification of the Tn-site, using simple and user-friendly techniques.

Results: a modified zeocin-selected HIMAR-1 transposon was placed in a mycobacterial phage. We also designed a Tn-seq system based on self-ligation, rather than adapter ligation. The system identifies the Tn-site based on 26 nucleotides, as compared to 14 nucleotides in the previously described system. We showed that in every given organism, the new system allows more sites to be identified unequivocally. Also, we create a Tn-mutant library in M. abscessus, with coverage of over 65% of the bacterial genes.

Summary: the new transposition system is effective highly stringent, and can be used in all mycobacteria. the Tn-seq system we developed is simply, effective and used friendly. These results were recently published in **mSystems**.

17

Genomic comparison of two strains of Mycobacterium avium subsp. paratuberculosis with contrasting pathogenic phenotype

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Abstract

In a previous study, we evaluated the degree of virulence and immune response of Map strains isolated from cattle in Argentina in a murine model. This assay allowed us to differentiate between high-virulent Map1347 and low-virulent Map1543 strains (Colombatti et al 2018).

The differences in virulence could be attributable to differences in the genomic sequences. We performed WGS and the comparison of the two genomes, retrieved 353 genes with at least SNPs/INDELS in one or both of the strains in relation to that of the Map K10 reference strain. The two strains share most of the variations. Only 14 of the mutations are present in one strain.

An in frame deletion of 12bp in Map_0403c gene, produces a deletion of four aminoacids in a putative protease homologous to the Rv 3671c gene in M. tuberculosis H37Rv in strain Map1347. The activity of Rv3671c protects M. tuberculosis against both acidic and oxidative stress encountered in the macrophage phagosome (Biswas et al, 2010). We performed in vitro experiments to evaluate the acidic and oxidative stress resistance of the two strains, compared to K10. Survival of Map strain 1347 in MDBM was higher than Map 1543. Moreover, Map1347 was more resistant to acidic pH and H_2O_2. Although we still need to evaluate if the deletion in Map_0403c gene in strain 1347 affects the structure of the protein and is responsible for the phenotype observed, Map_0403c is a good candidate to evaluate its role in the virulence of Map.

18

In Mycobacterium abscessus, the stringent factor Rel regulates metabolism but is not the only (p)ppGpp synthase.

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Abstract

The stringent response is a broadly conserved stress response system that exhibits functional variability across bacterial clades. Here, we characterize the role of the stringent factor Rel in the nontuberculous mycobacterial pathogen, Mycobacterium abscessus(Mab). We found that deletion of rel does not ablate (p)ppGpp synthesis and that rel does not provide a survival advantage in several stress conditions or in antibiotic treatment. Transcriptional data show that Rel(Mab) is involved in regulating expression of anabolism and growth genes in the stationary phase. However, it does not activate transcription of stress response or antibiotic resistance genes and actually represses transcription of many antibiotic resistance genes. This work shows that there is an unannotated (p)ppGppsynthetase in Mab.

19

Tetracycline destructase (TetX) from M. abscessus is not sufficient to induce tetracycline resistance in other mycobacteria.

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Abstract

Introduction: Mycobacterium abscessus is an environmental rapidly growing mycobacteria (RGM). The interest in recent years is due to its emergence as an important human pathogen. Not only is it the most prevalent RGM isolated in nontuberculous mycobacteria (NTM) infections in human, but is also famous for its drug resistance. In the case of tetracycline, M. abscessus was found to be 500-fold more resistant when compared with M. smegmatis and M. tuberculosis. According to common perception, it is related to efflux pumps and ribosome protection proteins; however, recent research demonstrated that the resistance in M. abscessus is attributed to WhiB7-independent tetracycline-inactivating monooxygenase, MabTetX (MAB_1496c).

Methods and results: Here, we aimed to test whether this MabTetX confers resistance to tetracycline in other mycobacteria when expressed there. MabTetX was cloned into a plasmid, and introduced into M. smegmatis. The recombinant strain, mDB268, was tested for its minimum inhibitory concentration (MIC). We found that mDB268 strain showed no increased resistance, with a MIC of 4 µg/ml, identical to WT. Overexpression of the gene from a multi-copy plasmid did not result in increased resistance either.

Conclusions: These results imply that the resistance mechanism to tetracycline is more complicated. MabTetX alone is not sufficient for induction of resistance, and additional factors in M. abscessus, absent from M. smegmatis, may be needed. Further understanding of the mechanism might be useful in the struggle against M. abscessus as well as in applications like selection markers.

20

The Effect of Ribosome Hibernation on Amikacin Tolerance in Mycobacterium abscessus

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Abstract

Mycobacterium abscessus (Mab) is an emerging pathogen among non-tuberculous mycobacteria characterized by intrinsic and acquired antibiotic resistance. Infections caused by Mab require a 18-24 month long multi-drug treatment regimen, highlighting the urgent need for the development of effective therapeutic strategy. The majority of chemotherapeutic approaches for Mab infection target the bacterial ribosome. This paradigm invites exploration into the physiological processes which confer Mab resistance to ribosome-targeting antibiotics, and illustrates the clinical relevance of such exploration. It was recently established that zinc starvation in Mycobacterium smegmatis, a closely related species to Mab, induces remodeling and hibernation of 70S ribosomes. Ribosome hibernation involves binding of mycobacterial protein Y (Mpy) on the ribosomal 30S subunit near the inter-subunit interface, encompassing the decoding center. Binding of Mpy stabilizes the ribosome in an inactive state, which also confers aminoglycoside tolerance to the organism. We therefore hypothesized that zinc-responsive induction of ribosome hibernation confers Mab tolerance to amikacin, a frontline aminoglycoside used against Mab infection. We addressed this hypothesis by investigating the effect of zinc starvation on Mpy-binding to ribosome in Mab, and studied the relationship between Mpy and aminoglycoside tolerance in Mab. We observed a conserved mechanism of Mpy binding to ribosomes in zinc-depleted Mab. Moreover, we demonstrate Mpy-dependent amikacin tolerance in Mab, which can arise from a direct interaction between Mpy and the ribosome.

21

Evidence from mycobacteria vs. the historical model of (peptidoglycan and) mechanism of action of β-lactam antibiotics

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Abstract

According to the historical paradigm, D,D-transpeptidases (also known as penicillin binding proteins, PBPs) are only involved in synthesis of peptidoglycan and are inhibited by β-lactam antibiotics. This paradigm also states that a distinct class of enzyme, β-lactamases, inactivate β-lactams. This paradigm has informed how β-lactams are used in the clinic. It teaches that only one β-lactam, that is most specific to the PBP of causative bacterium, should be used at a time to treat its infection. If necessary, β-lactamase inhibitor is to be included to protect β-lactam antibiotics from β-lactamases. We assessed if the historical paradigm accurately describes the PBPs, β-lactamases and their relationship with β-lactam antibiotics in mycobacteria. Two lines of evidence challenge the historical model. Contrary to the common belief, select PBPs exhibited β-lactamase activity comparable to or higher than the classical β-lactamase, BlaC. Additionally, select dual β-lactam combinations exhibited synergy against mycobacterial growth, providing direct evidence for using dual β-lactam combinations to treat mycobacterial infections.

22

Rapid emergence of dual resistance to amikacin and rifabutin in an in vitro evolution experiment of M. abscessus

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Abstract

Treatment of Mycobacterium abscessus infections is hampered by its intrinsic and acquired drug resistance, and relapse or re-infection occur frequently. Multidrug regimes with different antibiotic classes are recommended to prevent or slow down the emergence of resistance, relapse and consequently treatment failure. In other bacterial species it is demonstrated that persisters could promote the emergence of resistance. Persister cells comprise a fraction of a clonal bacterial population that survive antibiotic treatment at a concentration bactericidal towards their susceptible kin. Although antibiotic persistence is not well understood in M. abscessus, it has previously been linked to a non-replicating state and likely has an impact on treatment success.

The aim of the current study was to explore the mechanisms of persister formation in M. abscessus by identifying and characterizing genetic mutations affecting persister levels during in vitro evolution experiments to investigate their role in resistance development. For the latter, bacterial cultures were exposed to amikacin and rifabutin for 7 days, after which surviving cells were washed, passaged and treated again with the same antibiotic combination. Surprisingly, even under pressure of 2 antibiotics with different mechanisms of action, resistance rapidly emerged. Remarkably, amikacin resistance was acquired after 3 rounds of treatment, and amikacin resistant populations quickly developed resistance to rifabutin. This is in contrast to earlier reports involving Mycobacterium tuberculosis and the data indicates that M. abscessus may have different mechanisms to circumvent antibiotic killing, e.g., inducible macrolide resistance, which is consistent with its reputation for being refractory to antibiotic therapy.

23

Implications of mutations in a polysaccharide biosynthetic gene on Mycobacterium abscessus physiology and host-pathogen interactions.

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Abstract

Mycobacterium abscessus (Mabs), a group of opportunistic, rapidly growing mycobacteria, are known to cause infections in human lung, skin and soft tissue, as well as central nervous system. Relatively little is known of the strategies employed by Mabs to become a chronic pathogen of the Cystic Fibrosis lung. Recent studies have shown that Mabs undergoes pathogenic evolution in human hosts. The recent whole genome sequence analyses of serially isolated Mabs strains from a large population of CF patients identified the embC gene, mutating at a higher rate than would be expected by chance. The embC gene is involved in the biosynthesis of the major cell envelope polysaccharide, lipoarabinomannan, LAM. Six isogenic Mabs ATCC 19977 strains expressing clinically relevant embC mutations were generated. Preliminary results show phenotypic differences in the LAM structure that accompanied changes in the cell surface properties of the mutants as measured by sliding motility, congo red binding and biofilm forming capacity. Four of the six mutants were found to form smaller LAM as compared to the parent, wild-type, Mabs ATCC 19977. Using a biochemical approach, we hereby show that these mutations resulted in remarkable differences in the structure of LAM. This apparent difference may be predictive of differences in the way these isolates will interact with innate immune cells.

This work was supported by the Cystic Fibrosis Foundation.

24

Impact of clinically relevant ubiA mutations on the cell envelope heteropolysaccharides and cell surface properties of Mycobacteroides abscessus

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Abstract

Mycobacteroides abscessus (formerly Mycobacterium abscessus) is an emerging pathogen that has begun adapting to the human host. Whole genome sequencing of isolates from chronically infected patients revealed several genes were mutated at a higher rate than expected by chance that may be associated with host adaptation. One such gene, ubiA, is of interest. UbiA catalyzes the synthesis of the sole arabinose donor required to produce two major mycobacterial cell envelope heteropolysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM), which are necessary for envelope integrity and growth. Since bacteria primarily interact with the environment through the cell envelope, we hypothesize ubiA is under evolutionary pressure to enable M. abscessus adaptation to and persistence in the host by altering the cell envelope composition and its properties. Four isogenic strains expressing clinically relevant ubiA mutations were generated in M. abscessus subspecies abscessus ATCC 19977, a strain genetically similar to one of the dominant clones circulating worldwide. Biochemical analyses of the cell envelope mainly focused on AG and LAM. Cell envelope properties were measured by select assays including biofilm formation and sliding motility. Current results indicate AG and LAM abundances and structures are differentially altered depending on the mutation, with some mutations enhancing biofilm formation. Since we are mimicking clinically relevant ubiA mutations, these results suggest an altered profile of cell envelope heteropolysaccharides can facilitate M. abscessus drug tolerance and fitness for the human host, in part through enhancement of biofilm formation.

This work was supported by the Cystic Fibrosis Foundation.

25

Implementation of a mycobacterial CRISPRi platform in Mycobacterium abscessus and demonstration of the essentiality of $ftsZ_{Mab}$

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Abstract

Limited functional genetics tools in Mycobacterium abscessus hamper experimental validation and characterization of putative drug targets or resistance determinants. The implementation of CRISPR interference (CRISPRi) platform would greatly accelerate the elucidation of gene function in this emerging pathogen. In this study, we evaluated the mycobacterial single plasmid CRISPRi-dCas9Sth1 system for inducible gene silencing in M. abscessus by creating knockdowns in two well-characterized genes associated with antibiotic resistance (bla_{Mab}, $whiB7_{Mab}$) and two predicted essential genes ($ftsZ_{Mab}$, $topA_{Mab}$). Phenotype and mRNA levels were assessed after induction with low (200ng/ml) and high (10µg/ml) dose of the inducer anhydrotetracycline (ATc). Inducible repression of Bla_{Mab} and $WhiB7_{Mab}$ enhanced sensitivity to antibiotics, amoxicillin and clarithromycin, respectively with no dose-dependent change. The degree of transcriptional silencing and leakiness varied significantly between the two genes despite similar PAM strength. In contrast, application of CRISPRi to the two essential genes revealed ATc dose-dependent silencing and loss of viability for $ftsZ_{Mab}$, but not $topA_{Mab}$. Silencing of $ftsZ_{Mab}$ resulted in a dramatic 4-6 log decrease in cell viability representing the first validation of $ftsZ_{Mab}$ as an essential gene. We also demonstrated the feasibility of simultaneous CRISPRi-mediated silencing of multiple Mab target genes. Overall, we successfully exploited the mycobacterial CRISPRi-dCas9Sth1 platform in modulating specific gene expression in M. abscessus. However, variable gene silencing, effects of inducer dosing, and leakiness of CRISPRi knockdown that appear to be target-gene specific and discrepant with CRISPRi determinants established in M. tuberculosis and M. smegmatis warrants further evaluation and modification of the CRISPRi platform in M. abscessus.

26

Deciphering the Mycobacterium abscessus BlaRI regulon and regulation of β-lactam resistance

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Abstract

Mycobacterium abscessus's β-lactamase Bla_{Mab} has been described as constitutively expressed and induced by several stress conditions, which prompts questions about its regulation. Mab is resistant to most antibiotics, and identifying regulators of antibiotic resistance is critical in understanding pathogenesis. S. aureus's BlaRI system is a prototypical protease-mediated two-component system regulating β-lactamase blaZ. Transmembrane protein BlaR1 binds extracellular β-lactams, inducing autoproteolysis of an intracellular peptidase domain which cuts dimerized BlaI bound to the blaZ promoter. Mtb has blaRI homologs 1846c ($blaI_{Mtb}$) and 1845c ($blaR_{Mtb}$). The crystal structures $BlaI_{Mtb}$ and S. aureus BlaI have been demonstrated to be similar. Predicted to be a transcriptional regulator, $BlaI_{Mtb}$ binds to the promoter region of Mtb's β-lactamase, blaC and several other genes. The Mab genes Mab_2414c and Mab_2415c (named $blaR_{Mab}$ and $blaI_{Mab}$ in this study) are orthologs for $blaRI_{Mtb}$ and candidates for the regulators of bla_{Mab}. Three strains were constructed to study the regulon of $blaRI_{Mab}$: a knockout of $blaRI_{Mab}$ (MabΔblaRI), a complement with endogenous promoter (MabΔblaRI+blaRIc), and a complement overexpressing $blaI_{Mab}$ (MabΔblaRI+blaI_OE). To determine if blaMab was induced by various β-lactams, transcription of bla_{Mab} in MabΔblaRI was measured with qRT-PCR. To identify genes comprising the $blaRI_{Mab}$ regulon, RNAseq was performed on the aforementioned strains and compared to the parental Mab strain. While $blaRI_{Mab}$ does not regulate bla_{Mab}, transcriptome signatures indicate roles in the regulation of lipid transport, membrane biogenesis, and ATP synthesis. Investigating regulation by these proteins in Mab can elucidate the complex metabolism of this emerging pathogen.

27

Efflux pump activity as a linezolid resistance mechanism in Mycobacterium abscessus

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Abstract

Most first line antibiotics cannot be used to treat Mycobacterium abscessus infection, making therapy arduous and ineffective. For many antibiotics, M. abscessus shows resistance despite lacking any genetic polymorphisms that would be expected to confer resistance. This suggests that other mechanisms, such as low permeability to antibiotics or drug efflux, likely contribute to M. abscessus recalcitrance to treatment. Our approach focuses on efflux pumps as putative causative factors for resistance to linezolid, an effective antibiotic agent used with success in other mycobacterial infections. Linezolid is ineffective in many M. abscessus isolates, but resistant strains do not display consistent point mutations in linezolid target genes. We identified multiple clinical isolates showing a decrease in linezolid resistance after addition of efflux pump inhibitors. To determine whether efflux pumps in these linezolid resistant strains were responsible for the ineffectiveness of the drug, we expressed a selection of candidate efflux pumps cloned from the resistant strains into the linezolid susceptible model organism Mycobacterium smegmatis. Expression of one efflux pump, MAB_2736, led to a significant increase in resistance. Moreover, overexpression of MAB_2736 in multiple linezolid sensitive M. abscessus strains rendered every strain resistant to linezolid. Together these data suggest efflux pump activity is an underlying mechanism of linezolid resistance for many M. abscessus strains, highlighting the therapeutic potential of efflux pump inhibition in combination with linezolid for M. abscessus treatment.

28

Mycobacterium avium adapts during chronic cystic fibrosis infection through increased mutation rates leading to the acquisition of antibiotic resistance

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Abstract

Nontuberculous mycobacteria (NTM) have recently emerged as detrimental pathogens in the cystic fibrosis (CF) community with substantial diagnosis and treatment challenges. However, the mechanisms by which NTM adapts during chronic CF infection (i.e., increasing virulence, persistence, antibiotic resistance) are largely unknown. We sought to identify these mechanisms by conducting whole genome sequencing (WGS) on 24 longitudinal samples collected from a single CF patient with chronic Mycobacterium avium complex infection over five years. We constructed phylogenetic trees using RAxML and BEAST to assess the relationships between isolates and ClonalFrameML to estimate the recombination-to-mutation rate. Mutation rates were also estimated in vitro using fluctuation tests. We used liquid chromatography-mass spectrometry (LC-MS) to assess proteomic expression differences among isolates. We also examined important phenotypic properties such as colony morphology, sliding motility, biofilm formation, and antibiotic susceptibility. Our phylogenetic analysis revealed the presence of two distinct, coexisting populations of M. avium with a very recent common ancestor and fewer than 10 SNP differences in their core genomes. One lineage was characterized by high resistance to macrolide and aminoglycoside antibiotics and had mutations in the DNA repair and recombination genes, recR and uvrA. In addition, both phylogenetic and preliminary fluctuation analyses suggest that the antibiotic-resistant lineage has increased mutation rates compared to the susceptible population. Overall, this study highlights the role of hypermutability in bacterial adaptation during long-term CF infection.

29

Exploiting Multiplex CRISPRi to Identify and Prioritize Synergistic Drug Targets in Mycobacterium abscessus

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Abstract

Mycobacterium abscessus is emerging as a serious threat to cystic fibrosis (CF) patients. Compared to other non-tuberculous mycobacteria, Mab is more virulent, causing progressive decline of lung function and a higher fatality rate in CF patients. The success of this pathogen is attributable to its strong biofilm formation, highly impermeable cell wall, and notorious intrinsic resistance to many classes of antibiotics. Current treatment plans are often too lengthy and treatment failures occur in 50-70% of Mab cases, leaving physicians with no alternative but to perform lung resections. The much needed discovery and development of more efficient antibiotics requires validating new drug targets and prioritizing their vulnerability to inhibition and synergy. To address this, we are implementing CRISPRi to transcriptionally silence putative essential genes and gene combinations within Mab. Thus far, we have attempted to validate >10 Mab genes predicted to be essential through CRISPRi. Using the Golden GATEway cloning method, we will ligate sgRNA target sequences together to create multiplex CRISPRi constructs and therefore silence multiple genes simultaneously. In this study, two types of multiplex CRISPRi constructs will be evaluated: 1) sgRNAs targeting genes from the same pathway (e.g. protein synthesis); and 2) sgRNAs targeting genes from different pathways (e.g. protein synthesis plus cell wall). The performance of each multiplex CRISPRi construct will be evaluated based on the level of ATc required to reduce viability and the rate of bacterial killing. In future studies, optimized CRISPRi strains will be used to evaluate the vulnerability of drug target candidates in vivo.

30

Characterization of genetic requirements for Mycobacterium abscessus biofilm formation

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Abstract

Mycobacterium abscessus is a non-tuberculous mycobacterium (NTM) that causes opportunistic infections predominantly in the lungs of individuals with pre-existing lung diseases. M. abscessus infections are notoriously difficult to treat with less than a 50% cure rate. During infection, M. abscessus can persist as biofilm-like aggregates on the surface of the lung. Biofilms are communities of surface associated bacteria surrounded by a complex, polymeric extracellular matrix (ECM). Unlike single-cell planktonic bacteria traditionally studied in laboratory settings, biofilms allow for coordinated resource sharing and physical interactions, and even exhibit decreased sensitivity to antibiotics and antiseptics. How M. abscessus biofilm formation, matrix components, and biofilm genes contribute to the clinically observed multi-antibiotic resistance is poorly understood. We have adapted a previously established biofilm culturing system in starvation conditions to culture M. abscessus as biofilms. We have confirmed successful biofilm development by characterizing several biofilm ECM hallmarks using microscopy. With this culturing system, we conducted an arrayed genetic screen that identified several candidate genes that cause changes in biofilm morphology. We are investigating the role of these genes in biofilm formation, antibiotic susceptibility, and other functional biofilm metrics. This work will provide insights into how biofilm-specific genes contribute to antibiotic resistance in M. abscessus.

31

High-content analysis of drug-induced changes to intracellular architecture in mycobacteria

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Abstract

Highly complex cell wall assembly and asymmetric division patterns contribute to the ability of pathogenic mycobacteria to withstand drug exposure and cause disease. Similarly sophisticated, but less widely appreciated, is the intracellular architecture that drives growth and survival. We used automated high-throughput light microscopy to systematically explore subcellular structures and organization in multiple mycobacterial species. We screened the new Mycobacterial Systems Resource (MSR) imaging dataset of over 1,000 fluorescently-tagged conserved proteins in Mycobacterium smegmatis (Msm) for candidate strains with biologically interesting localization patterns. Using OMEGA2, an image analysis pipeline designed for mycobacteria, we acquired comprehensive, populational-level protein localization data for 28 candidate strains. To interrogate protein function and organization, we measured changes to fluorescent protein dynamics of these Msm mutants following treatment with sub-lethal doses of drugs with known mechanisms of action. Here, we report the differential outgrowth kinetics among these Msm mutants after drug exposure as well as concurrent lipid and DNA staining data that together reveal novel insights into the intracellular landscape of mycobacteria. Finally, high-throughput imaging and analysis of protein dynamics in fixed Mycobacterium abscessus cells raises new questions about differential growth and survival strategies across this genus.

32

Complete Genome Sequencing of Clinical Mycobacteria

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Abstract

NTM are opportunistic pathogens with innate resistant to many antimicrobials and the capability to cause persistent infection. Sequencing efforts are underway to better characterize the genomes of common NTM infecting species such as M. abscessus and M. avium. Complete genomes are important for tracking disease spread across patients and to better understand the genomic content of NTM isolates. When selecting a sequencing platform, there is a tradeoff between read length and accuracy. Illumina Next Generation Sequencing is highly accurate, but the reads tend to be short. Oxford Nanopore Technologies (ONT) is less accurate overall but can report on libraries with reads that are tens of thousands of bases in length. Both length and accuracy are imperative for complete genome annotation. Here, we used a hybrid approach to capitalize on the accuracy of Illumina and the read length of ONT to produce highly connected and accurate genome assemblies of seven clinical NTM isolates extracted from patients with CF. Four of the assemblies were successfully closed with no remaining gaps and three of these contained plasmids. We identified the isolates as M. abscessus (n=3), M. avium (n=2), M. chimaera (n=1), and M. intracellulare (n=1). Further, we show that by using complete patient-specific genomes as a reference we could detect genomic structural changes and SNPs across same-patient longitudinal samples. The research detailed here demonstrates how complete genome assemblies can be utilized to better understand the progression of patient infections, possibly providing insights into a treatment course to improve clinical outcomes.

33

High-density transposon mutagenesis in Mycobacterium abscessus identifies an essential penicillin-binding lipo-protein (PBP-lipo) involved in septal peptidoglycan synthesis and antibiotic sensitivity

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Abstract

Mycobacterium abscessus (Mab) is a rapidly growing non-tuberculous mycobacterium that causes a wide range of infections. Treatment of Mab infections is difficult because the bacterium is intrinsically resistant to many classes of antibiotics. Developing new and effective treatments against Mab requires a better understanding of the unique vulnerabilities that can be targeted for future drug development. To achieve this, we identified essential genes in Mab by conducting transposon-sequencing (TnSeq) on the reference strain, ATCC 19977. We generated ~51,000 unique mutants and used this high-density library to identify 362 genes essential for in vitro growth. To investigate species-specific vulnerabilities, we further characterized MAB_3167c, a predicted penicillin-binding-lipoprotein (PBP-lipo) that is essential in Mab and non-essential in M. tuberculosis (Mtb). We found that PBP-lipo primarily localizes to the septum as cells grow and prepare to divide. Knockdown of PBP-lipo causes cells to elongate, develop ectopic branches, and form multiple septa. Dual knockdown of PBP-lipo with PBPs, PbpB, DacB1, and a carboxypeptidase, MAB_0519 lead to synergistic growth arrest. In contrast, these genetic interactions were absent in the Mtb model organism, M. smegmatis, indicating that the PBP-lipo homologs between the two species exist in distinct genetic networks. Finally, repressing PBP-lipo sensitized the reference strain and 11 Mab clinical isolates to several classes of antibiotics, including the beta-lactams, ampicillin and amoxicillin by greater than 128-fold. Altogether, this study presents PBP-lipo as a key enzyme to study Mab specific processes in cell wall synthesis and importantly positions PBP-lipo as an attractive drug target to treat Mab infections.

34

Lineage analysis of Mycobacterium abscessus subsp. abscessus isolates from a treatment refractory infection reveals genomic adaptations over a five-year period

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Abstract

A longitudinal series of Mycobacterium abscessus (MAB) isolates was recovered from a young man with advanced cystic fibrosis (CF) related lung disease who underwent over three years of unsuccessful antibiotic treatment for a MAB lung infection. This infection was ultimately resolved with the addition of bacteriophage therapy. Genomic analysis spanned the first positive culture through culture conversion, enabling an in-depth evaluation of population dynamics and adaptations to treatment. Methods: A total of 40 isolates, collected over 1,800 days underwent whole genome sequencing. The isolate population was analyzed for single nucleotide polymorphisms, time-scaled phylogeny, mutation rate, and accessory genome composition. Results: Isolates recovered prior to antibiotic treatment were primarily clonal within the dominant circulating clone (DCC1) of MAB. With the initiation of antibiotic therapy, a population shift occurred with the emergence of adapted sublineages and evidence of parallel evolution of functional mutations across clades. With the initiation of phage therapy, the MAB population became genetically less diverse, and isolates of only one sublineage were recovered prior to sputum culture conversion to negative. Together, the isolate population demonstrated a mean substitution rate of 2.7×10^{-7} mutations/site/year, and the common ancestor was dated approximately 30 months prior to recovery of the first isolate. Pan genome analysis revealed a substantial reduction in accessory genes after antibiotic and phage treatment, consistent with selection for a persistent and homogeneous population. Conclusions: This unprecedented view of MAB airway infection simultaneously reveals the genetic stability of a MAB population and adaptive mutations resulting from sustained selection pressures.

35

Identification of molecular determinants that govern morphotype-specific physiology in Mycobacteroides abscessus

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Abstract

Little is known about the mechanisms underlying the physiological and behavioral differences between Mycobacteroides abscessus' morphotypes, smooth and rough. Although genetically similar, the morphotypes differ in antimicrobial tolerance, multicellular structures, immune activation, and patient disease progression. Transition from smooth to rough is due to loss of the hydrophilic surface-exposed glycopeptidolipid layer. It is unclear if this is the only driving force leading to phenotypic divergence. To investigate if the morphotypes possess other molecular mechanisms governing phenotypic differences, we generated transposon sequencing libraries in a ATCC19977 smooth and rough isolate. Although many essential genes are shared (441) under a nutrient-rich condition, smooth and rough MAB morphotypes have 165, and 59 unique essential genes, respectively. Further utilizing the Tn-seq libraries, we asked if the morphotypes utilize distinct genes for fitness during a skin abscess infection. Notably, the two morphotypes displayed more divergent essential genes compared to in vitro conditions with smooth requiring 138 unique genes, rough 109, and both requiring 137 genes not essential in vitro. These results indicate smooth elicits stress-induced DNA repair for in vivo fitness, while the rough requires several membrane proteins. By subjecting the libraries to several host-mimicking cues, we aim to deconvolve which and how each morphotype responds to specific cues during infection. Together, our findings show that smooth and rough morphotypes contain distinct genetic determinants underlying MAB fitness during infection and shed light on how the morphotypes differentially interact with the host.

36

The sRNA B11 controls virulence-associated phenotypes in Mycobacteroides abscessus

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Abstract

Little is known about the roles of small regulatory RNAs (sRNA) in Mycobacteroides abscessus. We found that deletion of the sRNA B11 in a smooth strain caused an intermediate smooth/rough morphology, increased antibiotic resistance, increased virulence in infection models, stronger innate immune activation, and increased transport to lysosomes. We identified several clinical isolates with B11 mutations. We used RNAseq to investigate the effects of B11 on gene expression and test the impact of two clinical mutations. ~230 genes were differentially expressed in ΔB11 compared to a complemented strain. Most of the genes differentially expressed in ΔB11 showed similar expression trends in strains with the clinical mutations, suggesting the clinical mutations caused partial loss-of-function. B11 has two C-rich loops previously found to repress expression in M. smegmatis by base-pairing to complementary sequences in ribosome binding sites (RBSs) of mRNAs. Among the genes upregulated in the ΔB11 mutant, there was a strong enrichment for the presence of B11-complementary RBSs. Comparing the proteomes of WT and ΔB11 strains likewise revealed a strong enrichment for B11-complementary RBSs in genes encoding upregulated proteins. Genes upregulated in ΔB11 included components of the virulence-associated ESX-4 secretion system. One of these had a B11-complementary RBS and fusing the RBS to a reporter made the reporter suppressible by B11. Together, our data show that B11 is a negative regulator with pleiotropic effects on gene expression and clinically important phenotypes in M. abscessus. To our knowledge, this is the first report of the role of an M. abscessus sRNA.

Pathogenic Strategies of NTM and Host Immune Response to NTM Infections

37

Phenotypic and genomic characterization of novel non tuberculous mycobacteria species

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Abstract

Apart from reporting for the first time the epidemiology of pulmonary infections with atypical mycobacteria in Tunisia, this study led to the identification of two new mycobacterial species, including one, baptized Mycobacterium fortunisiensis sp. Nov, seems to be pathogenic for humans and the other one seems to be ancestral with a potential of bioremediation. Based on multilocus sequencing, we have identified a putative novel rapidly growing Mycobacterium species, referred to as TNTM28, recovered from the sputum of an apparently immunocompetent young man with an underlying pulmonary disease. Here we provide a thorough characterization of TNTM28 genome sequence, which consists of one chromosome of 5,526,191 bp with a 67.3% G + C content, and a total of 5193 predicted coding sequences. Phylogenomic analyses revealed a deep-rooting relationship to the Mycobacterium fortuitum complex, thus suggesting a new taxonomic entity. TNTM28 was predicted to be a human pathogen with a probability of 0.804, reflecting the identification of several virulence factors, including export systems (Sec, Tat, and ESX), a nearly complete set of Mce proteins, toxin-antitoxins systems, and an extended range of other genes involved in intramacrophage replication and persistence. some of which had likely been acquired through horizontal gene transfer. Such an arsenal of potential virulence factors, along with an almost intact ESX-1 locus, might have significantly contributed to TNTM28 pathogenicity, as witnessed by its ability to replicate efficiently in macrophages. Overall, the identification of this new species as a potential human pathogen will help to broaden our understanding of mycobacterial pathogenesis.

38

Mycobacterium abscessus resists the innate cytotoxic response by surviving granzyme-mediated cell lysis of infected phagocytes

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Abstract

Mycobacterium abscessus, a human opportunistic pathogen, is the most pathogenic species among fast-growing mycobacteria, that are predominantly saprophytic. This is due to its ability to survive within the host, causing severe infections that are difficult to eradicate. How M. abscessus infects and colonizes the host and what could explain its pathogenicity remain poorly understood. Using primary murine cells and Drosophila, we demonstrate that intracellular M. abscessus remains insensitive to the granzyme-mediated cytolysis of infected phagocytic cells by cytotoxic cells. Indeed, we show the existence of such population in Drosophila, the thanacytes, which induce infected phagocytes apoptosis, depleting the main cell population capable of controlling the infection. M. abscessus systemic multiplication is thus favored since the observed antimicrobial peptides production is not required for the infection control. These results demonstrate the propensity of M. abscessus to resist the host's cytotoxic innate response, reminiscent of that observed with strict pathogenic slow-growing mycobacteria.

39

Characterization of Mycobacterium abscessus clinical isolates and prophage-encoded polymorphic toxins in an intramacrophage environment

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Abstract

Mycobacterium abscessus (Mabs) is a nontuberculous mycobacterial (NTM) species that contributes to the decline and death of patients with lung conditions such as cystic fibrosis (CF), bronchiectasis, and chronic obstructive pulmonary disease (COPD). Mabs infections are challenging to treat due to extensive drug resistance and its ability to survive intracellularly. There is wide genetic diversity among different Mabs strains with many carrying prophage encoded elements. Characterization of strains from diverse genetic backgrounds is required for a better understanding of Mabs pathogenesis. Towards this goal, we evaluated Mabs clinical isolates for intracellular survival. Intracellular survival was tracked by measuring Mabs colony forming units (CFU) in macrophage (THP-1) lysates over a time course. Of the four strains tested so far, all showed survival and a subset exhibited growth. We also constructed strains with and without prophage-encoded polymorphic toxin (PT) expression cassettes. Two PT expressing strains had the same intracellular survival phenotype as strains without PT cassettes. In order to further describe the Mabs intramacrophage environment, we determined if Mabs strains resided in acidified or non-acidified phagosomes using LysoTracker staining. Initial experiments indicate that phagosome acidification, an indicator of phagosome maturation, is dependent on strain morphotype. A smooth Mabs morphotype blocked phagosome acidification to a greater extent than a rough morphotype which lacks glycopeptidolipids in its outer membrane. The characterization of genetically diverse Mabs strains and prophage encoded genes will promote discovery of virulence factors required for intracellular growth and could lead to novel antibiotic targets.

40

Deciphering the contribution of different macrophage subsets to protection against pulmonary M. abscessus infection

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Abstract

Mycobacterium abscessus is a Non-Tuberculous Mycobacteria (NTM) that causes infection in individuals with compromised pulmonary immune responses. Chronic infection with M. abscessus is extremely difficult to eradicate and is associated with a significant decline in lung function in those infected. There is an unmet clinical need for novel prophylactic strategies to limit the burden of this pathogen, but the development of new vaccines has been limited by our incomplete understanding of immune correlates of protection against M. abscessus. This study used a resistant murine model of M. abscessus infection to characterise immune responses to pulmonary M. abscessus infection and identify the contribution of different macrophage subsets to protection. Following intranasal infection of C57BL/6 mice with a clinical isolate of M. abscessus, clodronate loaded liposomes were administered by intravenous or intranasal route to selectively deplete monocyte-derived macrophages or alveolar macrophages, respectively. At 7 days post infection, bacterial burden together with immune cell composition and phenotype was determined in lungs and lymphoid organs. While depletion of alveolar macrophages had no appreciable impact on bacterial burden, depletion of monocyte-derived macrophages resulted in a significant increase in bacterial burden in the spleen. This was coupled with a marked change in innate immune cell composition within the lung, in addition to adaptive immune subset accumulation, activation and phenotype. These findings indicate the importance of monocyte-derived macrophages in dictating the outcome of infection with M. abscessus, and highlight the need for continued characterisation of immune correlates of protection against this pathogen to enable effective vaccine design.

41

An Agent-Based Model to Assess the Effects of Biofilms and Repeat Exposure in Mycobacterium avium Colonization and Infection

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Abstract

Mycobacterium avium complex (MAC) pulmonary disease is caused by environmental microbes that are notoriously difficult to treat with both incidence and prevalence on the rise. We developed an agent-based computational model tracking interactions at the intracellular and tissue scale between bacteria, biofilm and immune cells in the lung airway. We hypothesize that biofilms are key to establishing infections and performed virtual biofilm knock-outs with our model. It is also known that patients regularly encounter MAC in common environmental sources and thus we explore host-pathogen dynamics by repeated exposure to bacteria.

We find that most biofilm is generated before bacterial deposition, rather than bacterial formation after inhalation. By knocking out biofilm that is deposited with bacteria, we see a significant decrease in total and infected macrophage counts and apoptosis at two weeks post-inhalation. Interestingly, there was no significant difference in total bacterial loads or bacteria that invaded the epithelium.

In preliminary experiments with repeated bacterial deposition, we see no significant difference in normalized bacterial loads remaining in the lung airway, but do see an increase in bacteria invading the epithelial layer and shifts in bacterial phenotypes relative to single exposure.

The decrease in macrophage counts and apoptosis in the absence of biofilms indicates that biofilms increase inflammation, but do not have an immediate effect on bacterial survival or invasion in the lung airway. Conversely, we do see an increase in bacterial invasion with repeat exposure to bacteria, indicating that decreasing environmental exposure, rather than biofilms, may have a preventative effect.

42

Acquisition of host-derived lipids by intracellular mycobacteria and its impact on pathogenesis

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Abstract

Intracellular mycobacteria predominantly rely on host-derived lipids such as sterols, phospholipids and fatty acids as carbon and energy sources during infection. This has been well described for Mycobacterium tuberculosis, but less is known about other pathogenic but non-tuberculous mycobacteria (NTM). Moreover, the mechanisms underlying the exact role of host-derived lipids in the intracellular life of mycobacteria remain to be explored. In this context, we propose to use Mycobacterium marinum together with the experimental host model Dictyostellium discoideum, well-established to study the interactions between intracellular pathogens and cell-autonomous defense mechanisms. First, using GFP-expressing bacteria, we demonstrated that M. marinum WT was able to grow using cholesterol, palmitate or oleate as main carbon sources in a dose-dependent manner. Genetic knock-out of systems involved in lipid import (Mce1/4) and utilization (LipY/Icl1/Facl6) led to growth alterations depending of the lipid and the dose used. These mutations also led to a significant intracellular growth defect in D. discoideum, while the initial capacity to infect cells was not altered, suggesting a limited ability to replicate. To understand this phenomenon, we initiated microscopy experiments aiming at characterizing the subcellular localization of these mutants affected in lipid acquisition. Preliminary results suggest that the ability to establish a replicative niche might be impaired for some of them. As perspectives, we aim to decipher the dynamics of host lipid availability during infection with M. marinum WT and the mutants, from the earliest stages of phagocytosis and intravacuolar growth, to escape to the cytosol and finally until their egress and dissemination.

43

Mechanical morphotype switching as an adaptive response in mycobacteria

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Abstract

The pathogen, Mycobacterium abscessus (Mab), causes severe infections requiring long antibiotic treatment regimens. Uncovering stress tolerance mechanisms rendering mycobacterial bacilli refractory to killing in adverse conditions of cidal stress during infection is critical for developing novel treatment methodologies. We developed long-term time-lapse atomic force microscopy (LTTL-AFM) for dynamic imaging at the nanoscale of cell mechanical properties influencing 1) the nature of fundamental cell processes for which we have no known molecular understanding and 2) the breadth of phenotypic heterogeneity. We identified that host macrophage intracellular M. smegmatis (Msm) and M. abscessus (Mab) both undergo “mechanical morphotype switching” as an adaptive mechanism influencing stress tolerance and optimizing rates of recovery. LTTL-AFM revealed that macrophages drive the selection of M. smegmatis bacilli that are either two-fold stiffer or softer than bacteria grown in axenic culture, reflecting two mechanically differentiated, semi-stable cell states. The “soft” mechanical morphotype exhibits higher rates of recovery than a “hard” mechanical morphotype. Enriching transposon mutants by buoyancy fractionation has revealed genes locking bacilli into a “soft” mechanical morphotype cell state. Soft mechanical morphotype mutants in both M. smegmatis and M. abscessus exhibited enhanced rates of recovery when liberated from macrophages, as compared to wildtype. Our findings suggest that mycobacteria possess the capacity to adapt into mechanically favorable cell states enhancing survival in adverse conditions. Our findings reveal a new paradigm for host-pathogen interactions and present unique opportunities to conceive of novel therapeutic strategies for driving mycobacterial pathogens into stress-sensitive states.

44

Investigating Sporulation Activity in Mycobacterial Strains

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Abstract

The Mycobacterium genus have evolved diverse mechanisms to survive harsh environmental conditions (nutrient deprivation, extreme temperatures, and drug selection pressure). Environmental bacteria such as Bacillus spp., the formation of endospores is a key survival mechanism harsh environmental conditions. Sporulation aids in disease transmission since pathogens can easily be dispersed in such state. Also, it is a mechanism by which Mycobacterium spp. can attain dormancy and persist in the environment and survive in the host. This study investigated sporulation in Mycobacterium spp., using Bacillus spp. as a control model, and profiled sporulation-associated markers. Growth profiles of Mycobacterium spp. exposed to diverse stress conditions (nutrient deprivation (OADC and glycerol), temperature (18˚C, 32˚C and 37˚C), hypochlorite (0.1 v/v, 0.01 v/v and stock) and UV (15 min)) were monitored for 8 weeks alongside the control (Bacillus spp.). Differential spore staining was performed to identify the presence of spores. Sporulation markers were profiled using primer-specific PCR amplification. Differential gene expression of sporulation associated markers at various stress conditions were determined using RT-qPCR. The Mycobacterial isolates formed spores relative to the Bacillus spp. Sixteen out of the twenty selected sporulation genes were detected in the strains. The sporulation markers were differentially expressed under the various stressed conditions. These findings suggest that sporulation is an adaptation mechanism used by mycobacteria to survive and persist in stressful environment. The formation of spores or spore-like structures could play a role in disease transmission and pathogenesis.

45

Induced synthesis of mycolactone restores the pathogenesis of Mycobacterium ulcerans in vitro and in vivo

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Abstract

Mycobacterium ulcerans is the causative agent of Buruli Ulcer. Virulent M. ulcerans secretes mycolactone, a polyketide toxin. M. ulcerans infection are mostly described as an extracellular milieu in the form of a necrotic ulcer. While some evidence exists of an intracellular lifecycle for M. ulcerans, the exact role that mycolactone plays in this process is poorly understood. Many previous studies have relied upon the addition of purified mycolactone to cell culture systems to study its role in M. ulcerans pathogenesis and host response modulation. However, this sterile system drastically simplifies the M. ulcerans infection model and assumes mycolactone is the only relevant virulence factor expressed by M. ulcerans. Here we show that the addition of purified mycolactone to macrophages during M. ulcerans infection overcomes the bacterial activation of the mechanistic Target of Rapamycin (mTOR) signaling pathway that plays a substantial role in regulating different cellular processes, including autophagy and apoptosis. To further study the role of mycolactone during M. ulcerans infection, we have developed an inducible mycolactone expression system. Utilizing the mycolactone deficient Mul::Tn118 strain that contains a transposon insertion in the putative beta-ketoacyl transferase (mup045), we have successfully restored mycolactone production by expressing mup045 in a tetracycline-inducible vector system. The inducible mycolactone expressing bacteria resulted in the establishment of infection in a murine footpad model of BU similar to that observed during the infection with wild-type M. ulcerans. This mycolactone inducible system will allow for further analysis of the roles and functions of mycolactone during M. ulcerans infection.

46

Immune Responses of C3HeB/FeJ mice to Environmental Mycobacterium chimaera

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Abstract

Mycobacterium chimaera is an emerging cause of nontuberculous mycobacterial (NTM) pulmonary disease associated with prior outbreaks in individuals undergoing open chest heart surgery via exposure to contaminated heater-cooler units. Little is known about the gradations in virulence among M. chimaera isolates. We previously identified a pair of environmental M. chimaera isolates, "M. chimaera 1" and "M. chimaera 2", categorized as genetically similar. Infection of these two isolates in either human THP-1 macrophages or primary human monocyte-derived macrophages consistently demonstrated greater recovery of M. chimaera 1. Herein, we infected C3HeB/FeJ mice to compare the in vivo immune responses to M. chimaera 1 versus M. chimaera 2. In contrast to the findings in macrophages, significantly lower counts of M. chimaera 1 were recovered from lung tissues and BAL cells with less overall lung histopathologic changes compared to mice infected with M. chimaera 2. Compared to M. chimaera 2, a more robust host-protective immune response, e.g., higher levels of IL-1a, IL-1b, IL-6, and TNF were quantified from lung tissue protein lysates early (Day 1) after M. chimaera 1 infection, which returned to baseline by Day 60. Analysis of metabolites in BAL fluid identified M. chimaera isolates differed in phospholipid and sphingolipid metabolism at tested timepoints. We conclude that the vigorous lung-specific immune responses to M. chimaera 1 may play a role in effective bacterial control, but for M. chimaera 2, subvert immune control. Continued studies of the gradations in virulence among the same species of NTM will advance our understanding of NTM pathogenesis.

47

Mycobacterium avium via oral route boosts BCG's protective efficacy against pulmonary tuberculosis: A central role of B-cell mediated immunity

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Abstract

Non-tuberculous mycobacteria (NTM) comprise a large group of mycobacteria that do not cause tuberculosis (TB) but are considered opportunistic pathogens and are ubiquitous in the environment. A critical concern regarding the development of an effective vaccine against TB is that most populations in developing countries are exposed to NTM daily; however, there is no precise information regarding the effect of NTM on the BCG's efficacy and TB immunity. Studies related to this subject remain challenging to interpret due to many variable experimental factors, including NTM concentration, route of exposure, the strain of NTM used, and BCG vaccination status. Considering the preceding, we developed a BCG+NTM mouse model that mimics the human BCG vaccination status (vaccination within one month of born) and NTM exposure (via drinking water). We found that BCG-NTM mice exhibited more robust and longer-term protection than BCG alone. These mice showed an increased number of B-cells and higher titers of IgA and IgG antibodies against Mycobacterium tuberculosis (Mtb) whole cell lysate before and after Mtb infection. Significantly, BCG-NTM mice also developed B-cell aggregates with properties of germinal centers, which are correlated with better protection and reduced pathology in the lungs. Our results provide strong evidence that a cross-protective relationship between NTM and Mtb exists, with B-cells playing a crucial role in this protection. Understanding the effect of NTM on vaccine efficacy will be a critical determinant of success in developing new vaccines for TB.

48

Discovery of nontuberculous mycobacteria associated with Kīlauea volcanic ash and their impacts on primary human macrophages and airway epithelial cells

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Abstract

In Hawai'i, the prevalence of NTM pulmonary disease is 4X higher than the national average, driven in part by island-specific environmental factors. The Kīlauea volcano is the world's most active and presents a unique respiratory hazard in Hawai'i. From volcanic ash collected during Kīlauea's 2018 eruption, we recovered viable Mycobacterium abscessus and Mycobacterium avium bound to Kīlauea ash (KA). We hypothesize KA antagonizes control of NTM infection by human immune cells. To assess the influence of volcanic ash on NTM growth in the absence of host cells, Kīlauea soil- or KA-derived NTM were incubated -/+ KA and changes in CFU were monitored up to 96hrs post infection. To determine if KA modulates NTM infectivity, monocyte derived macrophages (MDM) and alveolar macrophages (AM) from four healthy donors and airway epithelial cells cultured at the air-liquid interface (ALI) were infected with NTM -/+ KA and CFU tabulated up to 96hrs post infection. Cell supernatants were used to quantify IL-1B and to monitor cytotoxicity using LDH assays. KA showed low toxicity to NTM and lung cells. Significantly more M. abscessus were recovered from KA-exposed MDM 96hrs post infection compared to cells not exposed to KA ($p<0.05$). KA-exposed AM showed lower burden of KA-derived M. abscessus ($p<0.05$), but reduced control of KA-derived M. avium ($p<0.05$). KA-exposed ALI cultures demonstrated differential capacity control KA-derived NTM and control of NTM was independent of IL-1B secretion. While KA showed low toxicity, it may differentially influence immune cell control of NTM in a species dependent manner.

49

Recovery and characterization of novel environmental Mycobacterium abscessus rough morphotypes from Hawai'i

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Abstract

Mycobacterium abscessus (MABS) causes difficult to treat pulmonary disease. Previous reports suggest recovery of MABS from the environment is rare. To provide insights into the features of environmental MABS, we have microbiologically isolated viable MABS isolates from soil, dust, volcanic ash, freshwater biofilms, and streams from Hawai'i, a geographic hot spot for NTM pulmonary infections. It's surmised that the smooth morphotype is the infecting form found in the environment, which transitions into the more virulent rough morphotype in the host during the progression of disease, due to loss of glycopeptidolipids (GPL). Rough MABS has not been documented in the environment and MABS morphotypes have not been previously studied using large environmental isolate collections. We hypothesized that GPL increases survival of MABS in the environment to promote host infection. To elucidate the biology of environmental MABS morphotypes, we streaked 123 environmental Hawai'i MABS isolates onto 7H10 agar and assessed their morphotypes under a dissecting microscope. We found 97% (119/123) were smooth, 0.6% (1/123) were rough, and 2.4% (3/123) showed mixed morphotypes. From the three mixed samples, smooth and rough morphotypes were isolated (n=6 isolates) and assessed for GPL using thin layer chromatography and biofilms. We also compared survival of these environmental morphotypes in the presence of soil minerals, varying temperatures, phosphorus utilization, and survival in human macrophages and amoeba. To our knowledge, this is the first report confirming the existence of rough MABS in the environment. We continue to investigate the roles of environmental morphotypes on MABS pathogenesis.

50

Host-pathogen Interactions Between Healthy Alveolar Macrophages and Mycobacterium abscessus Dominant Circulating Clones Recovered from People with Cystic Fibrosis

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Abstract

Mycobacterium abscessus subsp. abscessus (MABS) is a significant pulmonary pathogen in people with cystic fibrosis (pwCF). We identified a panel of MABS dominant circulating clones (DCC1) and genetically diverse strains (non-DCC1) from pwCF who either met ATS criteria for NTM pulmonary disease (n=33) or did not meet these criteria (indolent infection; n=22), and evaluated the isolates for a range of in vitro phenotypes including morphology, pellicle formation, and presence of glycopeptidolipids (GPL). We also infected primary human alveolar macrophages (AM) from three healthy donors, and assessed MABS uptake and survival (CFU), and cytokine production (TNFα, MCP-1) over time. We hypothesized that in vitro phenotypes may explain the fitness of DCC1 isolates and predict clinical outcomes (NTM disease vs. indolent). DCC1 isolates were not associated with NTM pulmonary disease diagnosis and the binary in vitro phenotypes were not significantly associated with DCC1 genotypes or clinical outcomes. In contrast, MABS uptake and survival in macrophages was significantly higher for non-DCC1 compared to DCC1 isolates ($p<0.001$), and for GPL-positive isolates compared to GPL-negative isolates ($p<0.001$). Furthermore, non-DCC1 isolates from pwCF and NTM pulmonary disease showed higher CFU in macrophages over time compared to non-DCC1 isolates from pwCF and indolent infections ($p<0.01$). Increases in CFU over time were positively correlated with increased production of TNFα by infected cells ($p<0.001$). Our data suggest that MABS genotypes and intrinsic phenotypes such as cell wall lipid composition contribute to MABS infectivity and healthy host responses to infection, and may predict aspects of disease in at-risk patient cohorts.

51

The regulation of non-tuberculosis mycobacterial virulence genes in cystic fibrosis patients undergoing exacerbation treatment.

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Abstract

Introduction: Cystic fibrosis (CF) is an autosomal genetic disease that affects more than 70,000 people globally, and CF associated airway disease due to chronic infection is the leading cause of morbidity and mortality in this population. CF patients are more susceptible to lung infection and experience periods of exacerbation. Non-tuberculosis mycobacteria (NTM) have been detected in some populations of CF patients as an aetiological agent of lung infection. NTMs are opportunistic pathogens and are typically found in water sources, fomites, or surrounding environment. This study examines the regulation of mycobacterial virulence genes during periods of CF exacerbation. Method: Sputum samples were obtained from patients positive for NTM and experiencing exacerbation events during time of sampling. The control group were not taking any antibiotic therapy and the experimental group was on antibiotics. Patients were also matched using genotype, age and sex. Using two step RT-qPCR to measure gene expression, we examined the regulation of the genes responsible for erythromycin resistance (erm41), superoxide dismutase (sodA), heat-shock protein (hsp65) and RNA polymerase (rpoB). Results: The data was processed using the $\Delta\Delta$Ct method to measure the fold change in gene expression. This showed upregulation of all virulence factors across both groups but, the experimental group had higher levels of expression than the control. Conclusions: The upregulation of all virulence genes was higher in the experimental group than the control this could be due to the added pressure of the antibiotics forcing the NTMs to enable their defence and survival mechanisms.

52

Structural oddities of cell wall polysaccharides and their role in pathogenicity of Mycobacterium abscessus

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Abstract

The distinctive cell envelope of mycobacteria is a key modulator of their interactions with the host during infection. It contains a variety of glycoconjugates which structure, biosynthesis and biological importance has been studied in pathogenic mycobacteria, such as Mycobacterium tuberculosis, for decades but the same information for nontuberculous mycobacteria (NTM) is scarce. Increasing cases of infections caused by intrinsically resistant NTM to chemotherapeutic treatment call for deeper understanding of the physiology of these opportunistic pathogens.

Cell wall polysaccharide lipoarabinomannan (LAM) alongside with its biosynthetic precursors, phosphatidylinositol mannosides (PIMs) and lipomannan (LM), possesses crucial immunomodulatory properties in M. tuberculosis, but until now we have only assumed its structure and its role in the pathogenicity of NTM. As we report here, the structure of M. abscessus PIM, LM and LAM differs from those reported previously in other mycobacterial species in several respects including the presence of a methyl substituent on one of the mannosyl residues of PIMs as well as the PIM anchor of LM and LAM, the size and branching pattern of the mannan backbone of LM and LAM, and the modification of the arabinan domain of LAM with both succinyl and acetyl substituents. Investigations into the biological significance of some of these structural oddities point to the important role of polysaccharide succinylation on the ability of M. abscessus to enter and survive inside human macrophages and epithelial cells and validate cell envelope polysaccharides as important modulators of the virulence of this emerging pathogen.

53

Alpha-1-antitrypsin binds to the glucocorticoid receptor with anti-inflammatory and anti-mycobacterial significance in macrophages

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Abstract

Introduction: The anti-inflammatory effect of glucocorticoids (GC) can be lifesaving whereas its immunosuppressive effect increases vulnerability to infections. One mechanism by which GC cause both anti-inflammation and immunosuppression is by induction of apoptosis of lymphocytes. Alpha-1-antitrypsin (AAT) also has anti-inflammatory properties and yet enhances host-immunity against various viral and bacterial pathogens. While the glucocorticoid receptor (GR) mediates the pleiotropic function of GC, a canonical receptor for AAT has not been described. Since both GC and AAT have anti-inflammatory properties, we sought to determine if AAT binds GR and if so, whether this interaction has biological significance. Methods: we used co-immunoprecipitation, mass spectrometry, and microscale thermophoresis to determine if AAT binds GR. Results: in human macrophages, AAT co-immunoprecipitated with GR and vice versa. In addition, immunoprecipitation of GR, followed by mass spectrometry of the immunoprecipitated fraction demonstrated peptide sequences that were specific to AAT; in contrast, no peptide sequences matched to AAT upon immunoprecipitation with non-immune IgG. Microscale thermophoresis of fluorescent-labeled AAT with variable concentrations of GR revealed a differential shift in the movement of AAT in a thermal gradient, confirming that AAT binds GR in a cell-free system. In THP-1 cells stably knocked-down for GR by lentivirus-siRNA technology, AAT-inhibition of lipopolysaccharide-induced nuclear factor-kappa B activation and interleukin-8 expression as well as AAT inhibition of mycobacterial burden in macrophages were mediated by GR. Conclusion: AAT binding to GR mediates the anti-inflammatory and anti-mycobacterial effects of AAT.

54

Unravelling the pathogenicity of Mycobacterium abscessus clinical isolates in CF pulmonary epithelial cell and mouse models of respiratory infection

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Abstract

Mycobacterium abscessus (MA) infections in Cystic fibrosis (CF) patients display heterogeneous clinical outcomes. To date, the contribution to MA pulmonary disease (MA-PD) development by dominant circulating clones (DCCs) or morphotypes, remains to be elucidated. We aim at defining pathogenicity of CF MA clinical strains in CF pulmonary epithelial cell and mouse models of MA respiratory infection.

We collected eleven longitudinal MA strains isolated from five patients both at the early asymptomatic and MA-PD phase. We performed morphotype (rough and smooth phenotype) and whole genome sequencing (WGS) analysis. Moreover, we studied the host response induced by CF isolates in CF epithelial cells (CFF-16HBEgeCFTRΔF508) by host RNA sequencing and cytokines release. We also tested the virulence of MA clinical strains in mouse models of lung infection.

Epithelial cells infected with DCC1 strains displays a higher pro-inflammatory response than DCC2 strains. Moreover, we found out that morphotype (smooth vs rough strains) is the main bacterial feature driving over 3000 host differentially expressed genes. This was confirmed also by the evaluation of IL6 and IL8 protein levels upon infection. Then, we tested the in vivo pathogenicity of two longitudinal strains from the same patient, belonging to DCC1 and displaying a different morphotype. Longitudinal persisted rough strain displayed a higher bacterial burden and pro-inflammatory response, such as airway monocyte recruitment, than smooth strain in acute and chronic mouse models of lung infection.

Our findings suggest that rough DCC1 strains persisted within CF lung may cause more severe respiratory infections.

Supported by Italian_CF_foundation FFC#23_2020

55

Defining complex mechanistic interactions and responses by macrophages during Mycobacterium abscessus infection

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Abstract

Mycobacterium abscessus (MAB) is a highly antibiotic resistant, rapidly growing non-tuberculous mycobacterium that infects patients with chronic lung diseases, like cystic fibrosis. Phagocytosis by macrophages and subsequent release of cytokines is critical to neutralize and terminate invading pathogens. While interactions between MAB and macrophages contribute to its pathogenesis, how macrophages bind, phagocytose, and ultimately neutralize MAB during infection remains largely unknown. These interactions are further complicated by the ability of MAB to transition from a smooth to rough morphology during infection. We hypothesize that understanding key MAB-macrophage interactions will identify critical host targets that can be leveraged to prevent infection. To test this hypothesis, we developed new fluorescent reporters in smooth and rough MAB and optimized a range of macrophage assays to elucidate MAB-macrophage interactions both with and without antibiotic treatment. We used these tools to conduct a forward genetic screen using a genome-wide CRISPR-Cas9 knockout library in immortalized bone marrow derived macrophages to identify host pathways that contribute to MAB uptake four hours following infection. Our results show glycosaminoglycan (sGAG) synthesis in macrophages is required to efficiently take up smooth MAB during early infection. We are now testing how key regulators of sGAG biosynthesis, UGDH, B3GLCT, B4GALT7 and B3GAT3, influence macrophage interactions with smooth and rough MAB. Future work will determine how sGAG pathways modulate rough MAB uptake in both bone marrow-derived macrophages and a novel alveolar-like macrophage model with the goal of defining key mechanisms driving host-pathogen interactions in the lungs.

56

An Agent-Based Model to Assess the Contribution of Menopause-Associated Immunological Changes to Increased Risk of MAC Infection

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Abstract

The mechanistic reasons for the disproportionate number of Mycobacterium avium Complex (MAC) cases in post-menopausal women, compared to pre-menopausal, remain unclear. We have developed an agent-based model of the early interactions between bacteria and host immune cells in the lung airway to explore infection progression at both the intracellular and tissue scales. We generated simulations that represent pre- and post-menopausal patients by scaling model parameters for innate immune processes known to be disrupted post-menopause: macrophage recruitment, phagocytosis, and bacterial killing. Using this model, we can quantify risk of each of these individually or interplay among these.

In our post-menopausal simulations, we find a significant increase in bacterial loads across all three phenotypes (sessile, planktonic, and intracellular) and infected macrophages, and a decrease in healthy macrophages. This finding is contrasted by a decrease in total phagocytosis. Slowed macrophage phagocytosis leads to increases in extracellular bacteria. While decreased killing rates lead to increased accumulation of intracellular bacteria.

Taken together, these results suggest that menopause-associated parameters drive an overall increase in all bacterial phenotypes and an increase in the proportion of macrophages that are infected. The increase in infected macrophages post-menopause suggest that the accumulation of extracellular bacteria is enough to overcome impaired phagocytosis, while the higher intracellular bacteria levels indicate that bacterial killing is the limiting factor in post-menopausal patients' bacterial elimination. Thus, menopause could affect the balance between healthy and infected macrophages which, in turn, will have significant impacts as T-cells begin to respond.

57

Immunogenicity and protection against Mycobacterium avium with a heterologous RNA prime and protein boost vaccine regimen

<u>Susan Baldwin</u>[1], Valerie Reese[1], Sasha Larsen[1], Tiffany Pecor[1], Maham Rais[1], Hazem Abdelaal[1], Debora Ferede[1,2], Jesse Erasmus[3], Jacob Archer[3], Amit Khandhar[3], Brendan Podell[4], Steven Reed[3], Rhea Coler[1,2]

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Abstract

Pulmonary lung disease caused by nontuberculous mycobacteria (NTM) is becoming an increasing health threat. Toxic side-effects from anti-mycobacterial drugs can affect patient compliance, preventing the completion of drug treatment. A vaccine against pathogenic NTM, used as an adjunct to drug treatment, could reduce treatment time and lower the risks of severe side-effects.

Prophylactic efficacy of two delivery platforms for vaccination against Mycobacterium avium (M. avium) were tested. The vaccine antigen, ID91, includes 4 mycobacterial antigens: Rv3619, Rv2389, Rv3478, and Rv1886. ID91+GLA-SE is effective against a clinical NTM isolate, M. avium 2-151smt. Here we extend these results and show that a heterologous prime/boost strategy with repRNA-ID91 (replicating RNA) followed by protein ID91+GLA-SE boost is superior to the subunit-protein given as a homologous prime/boost regimen. The repRNA-ID91/ID91+GLA-SE regimen elicited CD4+ Th1 immune responses to four ID91 component proteins whereas the homologous protein prime/boost regimen induced responses to Rv1886 and Rv319 only. RepRNA-ID91/ID91+GLA-SE also induced TNF-secreting CD8+ T cells.

Finally, both vaccine regimens elicited protection against M. avium 2-151smt in Beige mice, measured by a significant decrease in bacterial load in the lung and spleen. Future studies are needed to determine whether these regimens induce long-lived memory immune responses or are protective against other NTM. Furthermore, we wish to test these vaccine regimens in the context of therapy as an adjunct to drug treatment.

Research was supported by the NIH under R21AI142267-01A1 and is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Poster Session 2 - Thursday June 2

3:15 - 5:00 pm

Intervention Strategies and Clinical Trials

58

Histopathology and treatment efficacy in a mouse model for pulmonary Mycobacteroides abscessus infection

Danielle Nicklas[1], *Emily Maggioncalda*[1], *Elizabeth Story-Toller*[1], *Gyanu Lamichhane*[1], *Randall Basaraba*[2]

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Abstract

Most notable among the nontuberculous mycobacteria is Mycobacteroides abscessus (Mab), an opportunistic environmental pathogen capable of causing chronic pulmonary infections in patients with structural lung conditions such as bronchiectasis, chronic obstructive pulmonary disease, and cystic fibrosis. Mab infections are often incurable with existing treatment recommendations and associated with rapid lung decline partially due to natural resistance to the majority of utilized antibiotics. Current treatment regimens include repurposed antibiotics, which are often associated with significant toxicities and side effects. Preclinical investigations into the nature of this emerging pathogen, in addition to the testing and development of novel antibiotic and regimens, remain stagnant largely due to a lack of proven mammalian models for Mab pathology.

Here, we demonstrate the utility of a recently developed mouse model of pulmonary Mab infection. In this model, corticosteroid treatment coupled with an aerosolized route of infection in immunocompetent mice permits initial proliferation and sustained pulmonary Mab infection that allows for pathological investigations in the lungs as well as the evaluation of efficacies of antibiotics. Implantation and sustained proliferation of pulmonary Mab evaluated through microbial burden over time show standard growth curve phases in vivo with both wild-type and clinical Mab isolates. Histopathology of murine lung lesions show immune infiltrates, high Mab burden, and organizing fibrosis consistent with defined granulomas of human Mab infections. Distinct bacteriostatic and bactericidal responses to different single-drug regimens, including the novel tetracycline derivative, omadacycline, and synergistic drug pairs showcase the utility of this model for preclinical testing of antimicrobial chemotherapies against Mab.

59

Optimization and characterization of AAPs (Nα-aroyl-N-aryl-phenylalanine amides) as potent RNAP inhibitors against NTMs and Mtb

Markus Lang[1]*, Lea Mann*[1]*, Uday S. Ganapathy*[2]*, Abdeldjalil Madani*[2]*, Thomas Dick*[2]*, Adrian Richter*[1]

[1]*Martin-Luther-Universität, Halle (Saale), Germany.* [2]*Hackensack Meridian Health, Nutley, NJ, USA*

Abstract

Infections with non-tuberculous mycobacteria (NTMs, e.g. M. abscessus) are on the rise (Dartois, Sizemore, and Dick 2019). Patients that have a compromised immune system or suffer from structural lung diseases like cystic fibrosis or COPD are prone to infections with these bacteria. NTMs possess a high level of intrinsic resistance against numerous antimycobacterial drugs (Lopeman et al. 2019) which makes drug development against these species of mycobacteria most urgent.

We investigate AAPs (Nα-aroyl-N-aryl-phenylalanine amides) as promising drug candidates that show high potency against different species of mycobacteria. The compound class targets the β-subunit of the bacterial RNAP. Starting with the hit MMV688845 from the Pathogen Box® library that was found to be active in a screen against M. abscessus (Jeong et al. 2018; Low et al. 2017; Richter et al. 2018) a panel of AAPs was synthesized that shows lower MICs against Mtb, M. abscessus and M. smegmatis indicating improved antimycobacterial activity. Even in infected macrophages derived from THP1 cells, the AAP derivatives retain activity against intracellular M. abscessus.

As the stereo configuration of AAPs is of importance for their activity, the reported synthetic pathway offers a low level of racemization making chiral separation unnecessary (Mann et al. 2021). Cytotoxic evaluation was found to be inconspicuous for different cell lines. A nephelometric solubility screen revealed that a few of the most active substances synthesized show improved solubility (up to 5 times better) compared to the original hit. This indicates promising pharmacokinetic properties for drug development and in vivo biopharmaceutical assessment.

60

Synthesis and derivatization of anti-TB hit compounds for characterization against M. abscessus

Paul Robin Palme[1], *Tom Schlegel*[1], *Lea Mann*[1], *Adrian Richter*[1], *Dereje Abate Negatu*[2], *Thomas Dick*[2], *Peter Imming*[1]

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Abstract

Infections with M. abscessus are not effectively treatable due to pronounced resistance to numerous classes of antibiotics. (Johansen et al., 2020) Therefore, new compounds need to be found.

Shirude et al. identified aminopyrazinamides as a hit against the GyrB ATPase of M. smegmatis. The optimization against M. tuberculosis led to development of the potent lead structure MMV687812 (MIC <0.5 µM against M. tuberculosis). (Shirude et al., 2013) Compound MMV687812 was active against M. abscessus (MIC80 12 µM). (Low et al., 2017) We corroborated activity of MMV687812 against M. abscessus in different in vitro assays and a MIC was determined against the fast growing mycobacterium M. smegmatis. A synthetic pathway was established and eight analogous were synthesized and characterized.

Squaramides (SQAs) described by Tantry et al. as specific and selective inhibitors of mycobacterial ATP synthesis are under investigation by us as NTM leads too. The most potent SQAs with a MIC 0.5 µM against M. tuberculosis were found to be efficacious in vivo and effective against bedaquiline resistant mycobacterial strains. SQAs are not cytotoxic against eukaryotic cell lines. (Tantry et al., 2017) Numerous active SQAs analogues are known to literature, but no activity determination against NTMs was performed. Therefore, several SQAs were synthesized and characterized against M. abscessus.

61

Novel Rifabutin Analogs Blocking Mycobacterial Drug Inactivation Exhibit Promising anti-Mycobacterium abscessus activity

Tian Lan[1], *Uday Ganapathy*[2], *Yong-Mo Ahn*[3], *Sachin Sharma*[1], *Matthew Zimmerman*[2], *Vadim Molodtsov*[3], *Richard Ebright*[3], *Veronique Dartois*[2], *Joel Freundlich*[3], *Thomas Dick*[2], *Courtney Aldrich*[1]

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Abstract

Mycobacterium abscessus (M. abscessus) has become a growing health threat towards the public, especially to patients with impaired immune systems or preexisting pulmonary diseases. The intrinsic resistance of M. abscessus to virtually all classes of existing antibiotics limits treatment options, and thus safe and effective therapeutics are urgently needed. Rifamycins, one of the most powerful sterilizing antimicrobials for treating mycobacterial infections, are up to 500-fold less active against M. abscessus due to an unprecedented drug inactivation mechanism by M. abscessus ADP-ribosyltransferase (Arr_{Mab}). Arr_{Mab} catalyzes site-specific ADP-ribosylation of rifamycins at the crucial C-23 position that is important for binding to rifamycin's target RNA polymerase β subunit (RPoB). In this work, rifabutin, one of the rifamycin antibiotics, was chemically derivatized at C-25 to produce an array of more than 30 analogs with diverse modifications. The synthetic analogs exhibited up to a 500-fold enhancement in their minimum inhibitory concentrations relative to rifampicin, with the best compounds demonstrating low nanomolar activity, commensurate with the activity of rifampicin against M. tuberculosis. Biochemical and structural studies confirmed that the most active analogs maintained their binding to the molecular target RPoB, but were completely resistant to Arr-mediated ADP-ribosylation. The most potent compounds also demonstrated superior activity against a panel of M. abscessus clinical isolates. Finally, the selected lead compounds were shown to possess low CYP3A4 induction and promising pharmacokinetic properties. Evaluation of in vivo efficacy in an M. abscessus-infected mouse model and further lead optimization are ongoing.

62

NBTI DNA gyrase inhibitors target Mycobacterium abscessus

Andreas Beuchel[1], Dereje A. Negatu[2,3], Abdeldjalil Madani[2], Matthew D. Zimmerman[2], Martin Gengenbacher[2,4], Véronique Dartois[2,4], Thomas Dick[2,4,5], Peter Imming[1]

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Abstract

Non-tuberculous mycobacteria, such as Mycobacterium abscessus, occur ubiquitously and can cause severe lung infections, most commonly affecting patients with pre-existing lung diseases such as COPD and cystic fibrosis. NTM are also a problem for livestock and wildlife and thus were included in One Health approaches. Considering these facts, the development of novel and broadly effective compounds against NTM is highly desirable.

In a screening of a library of small molecules containing compounds with known antibacterial activity against neglected infectious diseases (Pathogen Box®, Medicines for Malaria Venture), including TB agents, MMV688844 was identified to inhibit the growth of mycobacteria with bactericidal activity. We designed a series of derivatives of MMV688844 and tested them for their inhibitory activity against a variety of NTM species including Mycobacterium abscessus. From this series, we obtained candidates that exhibited a 10-fold increase in activity. In parallel, we also selected mutants of Mycobacterium abscessus that were resistant to MMV688844 and its derivatives. Genome sequencing of the mutants revealed that the compounds target mycobacterial DNA gyrase. The results suggest that the compound class represents a new chemical entity among the previously described novel bacterial topoisomerase inhibitors (NBTIs).

Subsequent investigation of the pharmacokinetic profile revealed general plasma instability of the compound class. Through iterative cycles of medicinal chemistry, we were able to generate a plasma stable compound that retained its antimycobacterial activity. On-going studies aim to further increase the potency of the stable compounds and decrease their hERG inhibitory activity, as the latter may be responsible for potential cardiotoxicity.

63

Synergy testing the RNA polymerase inhibitor MMV688845 against Mycobacterium abscessus

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Abstract

Mycobacterium abscessus (Mabs) is a non-tuberculous mycobacterium that is particularly difficult to treat. To eradicate such an infection, three or more antibiotics have to be used, depending on the resistance of the strain present, with healing success only emerging after years (Novosad et al., 2016). In addition, Mabs is able to grow intracellularly in human macrophages, or to form biofilms, which further increases the virulence of the pathogen (Wu et al., 2018). In order to test the efficacy of compounds in combination against Mabs, different assay models have been developed. We are using our established drug discovery assays to extensively characterize compounds such as Nα-2-thiophenoyl-d-phenylalanine-2-morpholinoanilide (MMV688845), which is a promising drug candidate (Richter et al., 2018; Mann et al., 2021). The compound was identified by screening of the Pathogen Box® library (Medicines for Malaria Ventures, MMV) against Mabs (Richter et al., 2018) and was further classified as RNA polymerase inhibitor (Lin et al., 2017). We use the "checkerboard" assay (Hsieh et al., 1993), which is applied for synergy testing and a fluorescence-based high-throughput assay developed for Mabs that allows efficient testing of compounds during macrophage infection (Richter et. al., 2020). Different assays are combined and performed, for example, checkerboard assays in the macrophage infection model. This allows us to detect the efficacy of substances under conditions that mirror the infection in the host. Under these conditions, we investigated the Substance MMV688845 to gain further insight into the potential of the compound and to advance its development into a drug substance.

64

Spray Dried Tigecycline Dry Powder Aerosols for the Treatment of Non-Tuberculous Mycobacteria

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Abstract

Tigecycline, a broad-spectrum, glycylcycline antibiotic, has demonstrated potential in treating patients with non-tuberculous mycobacterial infections. However, tigecycline's instability when reconstituted in aqueous solution and severe adverse reactions upon intravenous delivery have led to both technical challenges and poor patient compliance. Microparticle formulations of tigecycline for use in dry powder inhalers (DPI) represent an alternative delivery method to localize delivery to the major site of infection, mitigating off-target effects. Tigecycline was combined with lactose, to prevent tigecycline epimerization upon long-term storage, and phosphate buffer (pH 6.5), to maintain local physiological pH and increase patient tolerability. Subsequently, solutions were spray dried into respirable, corrugated, low-density microparticles. The ratio of tigecycyline:lactose:phosphate buffer salts spray dried was 90:0:10, 80:10:10, and 70:20:10, with the three amorphous powders having average particle geometric diameters of 1.8-1.9 µm and moisture content between 5.5 and 6.2 wt%. The emitted dose from a RS-01 DPI was 54.5 ± 6.7%, 56.3 ± 7.3%, and 60.8 ± 1.9% for 90:0:10, 80:10:10, and 70:20:10 tigecycline ratios, respectively. The mass median aerodynamic diameter for the formulations was between 2.7 and 3.0 µm, leading to fine particle fractions (particles with aerodynamic diameter < 5 µm) with respect to the emitted dose of 55-61%. While exhibiting promising aerodynamic properties, formulation optimization with the inclusion of leucine, an excipient often used to aid in particle dispersion, is likely to enhance the emitted dose and fine particle fraction metrics further and will be a topic of future research.

65

Omadacycline in M. abscessus Pulmonary Disease

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Abstract

Background Omadacycline has in vitro activity and in vivo efficacy in a mouse model against Mycobacterium abscessus complex (MABc). This Phase 2, double-blind, randomized, placebo-controlled, multicenter study evaluates the efficacy and safety of omadacycline in adults with MABc pulmonary disease (NCT04922554).

Methods Approximately 75 patients will be randomized 1.5:1 to 300 mg oral omadacycline (N=45) or placebo (N=30) daily for 84 days (D), stratified by prior use of antibiotics for MABc. Assessments occur at baseline, D14, D28, D56, D84 (end of treatment [EOT]), and D114 (safety follow-up). Inclusion criteria are age ≥ 18 years, diagnosis of MABc pulmonary disease per guideline criteria, ≥ 2 NTM symptoms at screening, ≥ 1 MABc-positive cultures within 6 months of screening and at screening, CT evidence of MABc within 3 months of screening, and guideline-directed antibiotic therapy not required within next 3 months.

Results Study objective is to explore the efficacy of omadacycline in patients with pulmonary MABc. The primary efficacy endpoint is: clinical response at D84 defined as improvement in severity of at least 50% of the symptoms present at baseline and no deterioration in severity of symptoms present at baseline. Secondary efficacy endpoints are: patient-reported outcomes, quality-of-life, and microbiological response from baseline to EOT. Adverse events (AEs) and serious AEs, laboratory parameters, vital signs and electrocardiograms will be monitored for safety.

Conclusion The trial began in June 2021 and will provide data on the safety profile, clinical response, patient reported outcomes and quality of life for omadacycline in patients with MABc pulmonary disease.

66

Analysis of the binding of the antimycobacterial imidazopyridine telacebec to the mycobacterial CIII2CIV2 respiratory supercomplex

Rana Abdelaziz[1], *David J Yanofsky*[2,3], *Justin M Di Trani*[2,3], *Sylwia Król*[4], *Stephanie A Bueler*[2], *Peter Brzezinski*[4], *John L Rubinstein*[2,3,5], *Peter Imming*[1]

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Abstract

Mycobacterial cellular respiration is a promising drug target. Telacebec, also known as Q203 (Pethe et al., 2013), is an imidazopyridine amide (IPA) that targets a large protein assembly in the electron transport chain known as the mycobacterial respiratory supercomplex III and IV (CIII2CIV2) or cytochrome bcc-aa3, which replaces the canonical CIII and CIV (Wiseman et al., 2018).

To understand the molecular mechanism of action and study the structure-activity relationship (SAR) of Q203, a 3D structure of the M. smegmatis CIII2CIV2 supercomplex free and bound to the inhibitor (Q203) was determined using cryo-electron microscopy (cryo-EM). Based on these structures, Q203 blocks two different menaquinol binding sites, inhibiting its oxidation, and thus preventing CIII2CIV2 activity. The atomic model of the supercomplex bound to Q203 was then used to study in silico the drug-protein interactions in the binding pocket. A halogen bond between the chlorine of Q203 and the carbonyl of Leu N:166 was found, a hydrogen bond between the N of the IPA and His M:368 and a pi-pi interaction between the phenyl group attached to the amide linker and Phe N:156 (Yanofsky et al., 2021).

Based on these findings, analogues with modified substituents were designed and synthesized for a more detailed study of the SAR. Through a 6-step synthetic scheme, analogues bearing heterocycles like benzoxazole were prepared. The side chain was chosen aiming to decrease lipophilicity and improve the solubility of the IPAs towards better uptake and biodistribution.

67

NF1001, a novel therapeutic option for NTM infections in CF patients.

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Abstract

NF1001 (FNDR-20364) is a novel, thiopeptide antibiotic produced by Streptomyces sps., isolated from Antarctica soil. It is highly potent, selective, bactericidal protein synthesis inhibitor active against mycobacteria, including non-tubercular mycobacteria (NTM) with an MIC range of 0.06µg/ml to 2µg/ml. NF1001 is also active against biofilms of NTM with 4X higher MICs.

The killing kinetics effect of NF1001 was better against the slow growing NTM (Mycobacterium avium (Mav) EmaxBiofilm=0.8 log10 cfu/ml vs. EmaxPlanktonic=1.43 log10 cfu/ml) compared to the fast growing one (Mycobacterium abscessus (Mabs) EmaxBiofilm=0.54 log10 cfu/ml vs. EmaxPlanktonic=1.1 log10 cfu/ml).

NF1001 is effective alone, and is synergistic in combination with SoC (Amikacin, Azithromycin, Rifampicin, Moxifloxacin) and the new drugs (Bedaquiline) against M. abscessus and M. avium biofilms. None of the combinations showed FICI >1.0, suggesting that NF1001 has a great potential to fit into the therapeutic SOC combination regimens.

NF1001, is equi-potent against drug resistant (MDR) clinical isolates of NTMs, resistant to protein synthesis inhibitors like Amikacin and Azithromycin, and the drugs targeting other vital mechanisms like Rifampicin, Moxifloxacin and Bedaquiline. This suggests that NF1001, though a protein synthesis inhibitor, yet has a unique MOA.

Thus, NF1001 has a potential to be developed as a candidate for treatment of hard-to-treat NTM infections.

68

Preclinical evaluation of apramycin as a drug candidate for Mycobacterium abscessus infections

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Abstract

Aminoglyocsides are an important component of mycobacterial therapy. Amikacin has been used in the treatment of MAC infections, but has shown lower potency against M. abscessus because of chromosomal resistance genes. Apramycin, a novel aminoglycoside antibiotic currently in clinical development for Gram-negative systemic infections, has demonstrated best-in-class activity against M. abscessus.

Here, we studied the activity, synergy, and time-kill kinetics of apramycin in comparison to amikacin. The in-vivo efficacy of apramycin was assessed in M. abscessus mouse infection models.

The MIC of apramycin was found to be 0.5 – 2 µg/mL in a panel of contemporary clinical M. abscessus isolates and thus about 4-fold lower than the MICs of amikacin. Checkerboard assays demonstrated additive antibacterial activity of apramycin when combined with bedaquiline or other drugs. In-vitro time-kill kinetics suggested a higher bactericidal potency of apramycin than of amikacin. A dose of 16 mg/kg apramycin resulted in CFU reductions in both SCID and CFTR/CFTR mice. In comparison, treatment with 16 mg/kg of amikacin had no significant effect on the CFU counts in CFTR/CFTR mice relative to the vehicle group. A mid dose of 64 mg/kg apramycin resulted in >1-log CFU reductions in both the SCID and the CFTR/CFTR model relative to the start of treatment.

The higher in-vitro activity of apramycin against M. abscessus translated into a more potent CFU reduction in infected mice than amikacin. Our findings warrant continued consideration of apramycin as a potential drug candidate for improved treatment regimens for pulmonary NTM infections.

69

Efficacy of epetraborole against Mycobacterium abscessus is increased with norvaline

Jaryd Sullivan[1], *Andréanne Lupien*[2], *Elias Kalthoff*[3], *Claire Hamela*[4], *Lorne Taylor*[2], *Kim Munro*[3], *Martin Schmeing*[3], *Laurent Kremer*[4], *Marcel Behr*[1]

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Abstract

Mycobacterium abscessus causes difficult-to-treat pulmonary infections in patients with impaired lung function. Cure rates are 50% or less and can be partially attributed to the paucity of effective antimicrobials against M. abscessus. Here, we identified epetraborole as an inhibitor of leucyl-tRNA synthetase (LeuRS) that does not have self-inducing resistance and protects zebrafish from lethal M. abscessus infection. After identifying $LeuRS^{D436H}$ mutants resistant to epetraborole, we reported the observation that norvaline, a non-proteinogenic amino acid, inhibited the growth of these mutants and resulted in the incorporation of norvaline across the proteome. Misaminoacylation of proteins with norvaline upregulated the unfolded protein response with GroEL chaperonins and Clp proteases. This supported our in vitro data that supplementing epetraborole with norvaline reduced the emergence of epetraborole mutants in both M. abscessus and M. tuberculosis and improved the efficacy of epetraborole in a murine model of M. abscessus infection. Our results emphasize the effectiveness of epetraborole against the clinically relevant pathogen M. abscessus, and these findings also suggest norvaline adjunct therapy with epetraborole could be beneficial for M. abscessus and other mycobacterial infections like tuberculosis.

70

New leucyl-tRNA synthetase inhibitor DS86760016 is active against Mycobacterium abscessus

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Abstract

Epetraborole, an advanced nonhalogenated 3-aminomethyl benzoxaborole is a new class of leucyl-tRNA synthetase (LeuRS) inhibitors and it was recently reported as effective inhibitor against Mycobacterium abscessus. However, based on ClinicalTrials.gov in 2017, a clinical phase II study of epetraborole for the treatment of complicated urinary tract infection and complicated intra-abdominal infection was terminated due to the rapid emergence of drug resistance during treatment. Recently however, a new oxaborole inhibitor, DS86760016, has reignited the re-use of the LeuRS inhibitor resulting in a lower frequency of resistance development than epetraborole in comparative murine urinary tract infection models. Unfortunately, DS86760016 is not commercially available currently because Daiichi Sankyo India, the pharmaceutical company that discovered DS86760016, was closed in 2017 (personal communication with Dr. Nobuhisa Masuda). For this reason, we synthesized DS86760016 and tested its activity against M. abscessus in vitro and in zebrafish infection / treatment model. In this study, we demonstrate that DS86760016 shows excellent in vitro and in vivo activity similar with epetraborole and it exhibited lower mutant frequency than epetraborole. Our findings confirm that DS86760016 can be advanced lead candidate of a benzoxaborole for the treatment of M. abscessus lung disease.

71

Efficacy of MmpL3 inhibitor CRS0393 against M. abscessus in a mouse model

Gyanu Lamichhane[1], *Chandra Panthi*[1], *Binayak Rimal*[1], *Teresa Hoang*[2], *Hang Liu*[2], *Cliff Mason*[2], *Thale Jarvis*[2], *Mary Ann DeGroote*[2], *Urs Ochsner*[2], *Xicheng Sun*[2]

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Abstract

Background: Regimens in the recommendations for treating M. abscessus (Mab) disease include repurposed antibiotics that are approved for other indications. The minimum inhibitory concentration (MIC) of most antibiotics available today vs. Mab is significantly higher than what is achievable in human serum and hence Mab is considered intrinsically resistant to most antibiotics. Therefore, the need to pursue agents that inhibit new targets in Mab with physiologically relevant MICs has been prioritized in drug discovery/development efforts. CRS0393 is one such candidate as it inhibits MmpL3, a transmembrane channel protein.

Methods: We assessed the efficacy of CRS0393 vs. three independent Mab isolates. C3HeB/FeJ mice were infected with aerosolized suspensions of Mab and received anti-inflammatory/immunosuppressing agent dexamethasone throughout the study duration. CRS0393 was administered via oral and intranasal. Mice treated with vehicle only and with a standard-of-care antibiotic were included as negative and positive control comparators. CFU counts were obtained at 1, 2, and 4 weeks. Lung sections were assessed for histopathology.

Results: CRS0393 delivered intranasally reduced lung burden of Mab CSU103 by >3 log10 and to the same level as that produced by the positive control comparator clofazimine. Although orally delivered CRS393 produced ~1 log10 reduction in lung burden of all three strains of Mab over a 4-week treatment period compared to untreated group, this reduction was not highly statistically significant.

Discussion: Variable efficacy in the dexamethasone-treated mouse model of Mab lung infection and a delayed response to CRS0393 suggest that treatment beyond 4 weeks may be needed.

72

Therapeutic efficacy of antimalarial drugs targeting DosRS signaling in Mycobacterium abscessus

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Abstract

Rapidly-growing nontuberculous mycobacteria (NTM) of the Mycobacterium abscessus complex have emerged as important human pathogens globally, and are linked to an increasing number of pulmonary infections among patients with structural lung disease such as chronic obstructive pulmonary disease, bronchiectasis, and cystic fibrosis. The low cure rate of currently available treatment regimens, in spite of a minimum of 12 months of chemotherapy, notable side effects, and frequent bacterial re-emergence associated with these regimens highlights the need for alternative approaches to treat NTM infections. A search for alternative M. abscessus treatments led to our interest in the two-component regulator DosRS, which, in Mycobacterium tuberculosis, is required for the bacterium to establish a state of nonreplicating, drug-tolerant persistence in response to a variety of host stresses. We show here that the genetic disruption of dosRS impairs the adaptation of M. abscessus to hypoxia, resulting in decreased bacterial survival after oxygen depletion, reduced tolerance to a number of antibiotics in vitro and in vivo, and the inhibition of biofilm formation.

We determined that three antimalarial drugs or drug candidates, artemisinin, OZ277, and OZ439, can target DosS-mediated hypoxic signaling in M. abscessus and recapitulate the phenotypic effects of genetically disrupting dosS. OZ439 displayed bactericidal activity comparable to standard-of-care antibiotics in chronically infected mice, in addition to potentiating the activity of antibiotics used in combination. The identification of antimalarial drugs as potent inhibitors and adjunct inhibitors of M. abscessus in vivo offers repurposing opportunities that could have an immediate impact in the clinic.

73

In vitro Activity and Mode of Action of CRS0393, a Potent MmpL3 Inhibitor as a Novel Antimycobacterial Agent

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Abstract

Background: Mycobacterial pathogens are intrinsically resistant to many antibiotics and pose a health threat particularly for susceptible individuals with cystic fibrosis (CF), chronic obstructive pulmonary disease, and bronchiectasis. Treatment options are often limited and suboptimal. There is an unmet need for new and better antimycobacterial agents. Extensive medicinal chemistry optimization has led to CRS0393, a novel benzothiazole amide with potent activity against NTM.

Materials/methods: Traditional medicinal chemistry approaches were used for structure-activity relationship exploration and compound optimization. NTM strains included reference strains and clinical isolates and were tested by CLSI methods.

Results: CRS0393 demonstrated excellent in vitro activity against rapid-grower NTM, including CF isolates, with minimum inhibitory concentrations (MICs) of ≤0.03 - 0.5 µg/mL. Good activity was also observed against M. avium and M. tuberculosis. Spontaneous resistant M. abscessus mutants selected at 4x MIC emerged with a frequency of 10^{-9} and carried specific mutations in the mmpL3 gene that encodes an inner membrane transporter essential for cell wall biosynthesis. MmpL3 was confirmed as the target via metabolic labeling using [^{14}C]-acetate, which showed that CRS0393 inhibited the transfer of mycolic acids to their cell envelope acceptors arabinogalactan and trehalose monomycolate (TMM). Furthermore, CRS0393 demonstrated concentration-dependent displacement of MmpL3-specific fluorescent probe North-114.

Conclusions: CRS0393 shows great potential as novel class of antimycobacterial agents that is not affected by preexisting resistance. Since the target, MmpL3, is found only in mycobacteria, CRS0393 is unlikely to cause disruption of normal human microbiota.

74

Therapeutic potential of dimethyl malonate against Mycobacterium avium complex infection by the modulation of β-oxidation

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Abstract

Mycobacterium avium complex (MAC) pulmonary disease has been increasing worldwide. The genus Mycobacterium are thought to be intracellular pathogens capable of regulating host metabolism, eventually affecting treatment outcomes as well as intracellular growth of MAC. We employed dimethyl malonate (DMM), a derivative of the malonic acid and an inhibitor of succinate dehydrogenase in tricarboxylic acid (TCA) cycle, to investigate whether altered host metabolism affect treatment outcomes and MAC growth inside macrophages. Surprisingly, the blockade of TCA cycle by DMM enhanced anti-MAC activity of macrophages. Transcriptomic analysis revealed that acyl-CoA thioesterase I, a regulator of the mitochondrial β-oxidation, was crucially involved in this improved anti-MAC activity of macrophages. Collectively, our proof-of-concept study suggested counter-regulation of host lipid metabolism regulated by MAC is a promising therapeutic target to control MAC infection effectively.

75

A clofazimine-containing regimen improves treatment outcomes in a chronic pulmonary Mycobacterium avium infection murine model

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Abstract

Treatment outcomes using the standard regimen (a macrolide, ethambutol, and rifampicin) for Mycobacterium avium complex-pulmonary disease (MAC-PD) remain unsatisfactory. Thus, improved treatment regimens for MAC-PD are required. Clofazimine has recently been revisited as an effective drug against mycobacterial infection. We performed a comparison between the standard regimen and an alternative regimen (replacing the rifampicin with clofazimine) based on the intracellular anti-MAC activities of the individual drugs in a murine model of chronic progressive MAC-pulmonary infection (MAC-PI). The intracellular anti-MAC activities of the individual drugs and their combinations in murine bone marrow-derived macrophages (BMDMs) were determined. The treatment efficacies of the standard and clofazimine-containing regimens were evaluated in mice chronically infected with M. avium (Mav) by initiating 2- and 4-week treatment at 8 weeks post-infection. Bacterial loads in the lung, spleen, and liver were assessed along with lung inflammation. Insufficient intracellular anti-MAC activity of rifampicin in BMDMs was recorded despite its low in vitro minimum inhibitory concentrations (MICs), whereas optimal intracellular killing activity against all tested MAC strains was achieved with clofazimine. Compared to the standard regimen, the clofazimine-containing regimen significantly reduced CFUs in all organs and achieved marked reductions in lung inflammation. The replacement of rifampicin with clofazimine in the treatment regimen resulted in more favorable outcomes in an animal model of chronic progressive Mav-PI. Intriguingly, 2 weeks of treatment with the clofazimine-containing regimen reduced bacterial loads more effectively than 4 weeks of treatment with the standard regimen in Mav-infected mice. Thus, the clofazimine-containing regimen also had a treatment-shortening effect.

76

Evaluation of antimicrobial activity of novel thiopeptide-derived antibiotics against Mycobacterium avium complex

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Abstract

Unsatisfactory treatment outcomes of the standard regimen (macrolide, ethambutol, and rifampicin) in patients with Mycobacterium avium complex (MAC) pulmonary disease have urged us to develop novel antibiotics. Thiopeptides, a class of peptide antibiotics derived from natural products, are one of promising drug candidates by targeting bacterial ribosomes, but drug development has been obstructed due to their extremely poor solubility. Here, we evaluated three compounds (AJ037, AJ039, and AJ206) derived from thiopeptide micrococcin P2 (MP2) by enhancing aqueous solubility based on structure-activity relationship analysis. We conducted in vitro drug susceptibility testing and intracellular activities of three MP2 derivatives against various MAC clinical isolates. Among tested drugs, AJ039 had the lowest MIC value (0.125 μg/ml) against MAC followed by AJ037 (0.25 μg/ml) and AJ206 (0.5 μg/ml). In addition, the compounds had a similar intracellular antimicrobial activity to clarithromycin (CLR), a core drug for MAC-PD treatment. Moreover, synergistic effects of these thiopeptide drugs with CLR on inhibiting MAC growth inside macrophages were found when compared with the use of individual drugs alone. Our current study suggests that AJ037, AJ039, and AJ206 can be promising anti-MAC agents for the treatment of MAC infection and further in vivo evaluation will warrant their attainable development.

77

Pharmacokinetics of a Novel Antimycobacterial Agent, CRS0393, after Oral and Intratracheal Administration in Mice

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Abstract

CRS0393 is a novel narrow spectrum antibacterial agent with excellent activity against clinically relevant mycobacterial species, including M. abscessus (Mabs), M. avium, and M. intracellulare. We explored feasibility and pharmacokinetics of intravenous (IV), oral (PO) and intratracheal (IT) administration of CRS0393 using specifically designed formulations for each route of delivery.

GM-CSF knockout mice and C57BL/6 mice were used in the evaluation of pulmonary and systemic pharmacokinetics. For IV and PO administration, CRS0393 was formulated in either kolliphor oil, HPβCD solution, or PEG400. Cohorts of three animals were sacrificed at each time point and blood, BAL fluid, and lung tissue were collected. Drug concentrations were measured by LC-MS/MS and analyzed by noncompartmental pharmacokinetic analysis.

Intratracheal instillation of a single dose of CRS0393 resulted in high concentrations of drug in ELF and lung tissue, which remained above the Mabs MIC range for at least 9 hours post-dose. This exposure resulted in a penetration ratio of 261 for ELF and 54 for lung tissue relative to plasma. The oral pharmacokinetics varied depending on CRS0393 formulation. The oral bioavailability ranged from 18 to >100% and was highest for drug formulated in kolliphor oil.

CRS0393 administered IT has good penetration in lung and ELF, thus underlining its suitability for the treatment of respiratory tract infections. Additionally, the demonstration of oral bioavailability indicates potential for oral treatment options. Further data suggests that in vivo efficacy of CRS0393 may be dependent on the route of administration and formulation as well as the animal model under study.

78

High-throughput screening of St. Jude Children's Research Hospital compound libraries reveal promising candidates for Mycobacterium abscessus therapy

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Abstract

In recent years, non-tuberculous mycobacteria (NTM) infections have grown more common, in some countries even surpassing the incidence of tuberculosis. Mycobacterium abscessus is among the most common causes of NTM infection, afflicting individuals regardless of immune status. Along with several other NTM species, M. abscessus is difficult to treat due to its intrinsic and adapted resistance to several classes of antibiotics. Antimicrobial resistance leads to unfavorable outcomes, thus demonstrating the urgent medical need to develop more effective, broad-spectrum antimycobacterial regimens. One strategy to overcome this intrinsic drug resistance and create effective treatment is to discover novel chemical matter that avoids resistance mechanisms. The primary objective of this study is to identify small molecules from chemical libraries provided by St. Jude Children's Research Hospital that are active against NTM species and characterize their modes of action. Thus far, over 9,000 compounds from this set have been screened in a luminescence-based high-throughput assay against M. abscessus ATCC 19977, generating a 2.74% hit rate including 272 compounds with >50% activity. On-going studies include screening a larger subset of compounds with drug-like properties and prioritizing hit compounds based on dose-dependent activity. These hit compounds will then be tested against a panel of NTM species and clinical isolates to evaluate their broad-spectrum antimycobacterial activity. Hits will be further evaluated for target identification, structural derivation, and biochemical and biophysical characterization. Ultimately, the results from this screening campaign will serve as a starting point for further development of small-molecule compounds that target NTM species.

79

Spectinomycin Analogs for the Treatment of Mycobacterium abscessus Infections

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Abstract

Non-tuberculous mycobacteria (NTM) are emerging pathogens with high intrinsic drug resistance. Among rapidly growing NTM species, Mycobacterium abscessus is among the most pathogenic. Standard of care therapy has led to unacceptable outcomes and demonstrates the urgent need to develop effective, broad-spectrum antimycobacterial regimens. To address this challenge, we investigated the synthetic modification of spectinomycin (SPC), an aminocyclitol antibiotic that exhibits potent protein synthesis inhibition, but has limited efficacy due to intrinsic whiB7-mediated resistance mechanisms. An extensive library (>300) of semi-synthetic SPC analogs were profiled for mycobacterial ribosome inhibition and in vitro activity against M. abscessus, from which a distinct structural subclass of ethylene linked aminomethyl SPC (eAmSPC) was identified. Initial eAmSPC leads display potent anti-M. abscessus activity, while maintaining desirable pharmacological properties. Their mode of mycobacterial ribosomal inhibition was confirmed by the generation of resistant mutants that track to the distinct helix-34 SPC binding site. Transcriptional profiling revealed that eAmSPC induces the whiB7 regulon similarly to SPC, however, eAmSPC retain potency in comparison to SPC regardless of WhiB7 status as determined by susceptibility testing against a WhiB7-deficient strain of M. abscessus. Further, eAmSPC display increased intracellular accumulation compared to SPC, indicating accumulation is the key for improved efficacy of the eAmSPCs. Lead eAmSPC also retain activity against multi-drug resistant M. abscessus clinical isolates and demonstrate robust efficacy in M. abscessus mouse infection models. The results of these studies suggest that eAmSPCs have the potential to be developed into clinical treatments for M. abscessus and other NTM infections.

80

The versatility of fluorescent probes to unravel efflux systems in Mycobacteria

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Abstract

Efflux pumps play a significant role in the intrinsic resistance of Mycobacteria because of their ability to extrude antibiotics allowing the bacterium to persist while gaining mutations. Thus, efflux pumps are a potential target to overcome intrinsic resistance and rescue antibacterial activity. In Mycobacterium tuberculosis, the efflux pump Rv1258c extrudes several antibiotics, including spectinomycin (SPC) and spectinamides. However, spectinamides with a 2-pyridine ring overcome Rv1258c-mediated efflux. Considering the structural similarities among the spectinamides, we hypothesize that low efflux spectinamides act as inhibitors of Rv1258c-mediated efflux. To test this hypothesis, we developed a series of Bodipy- and TAMRA-conjugated SPC and spectinamides to act as chemical probes to investigate Rv1258c-mediated efflux. Conjugation of Bodipy and TAMRA did not alter Rv1258c-mediated efflux and maintained differential susceptibility in M. tuberculosis. We validated the Rv1258c-mediated efflux activity of probes using accumulation and efflux assays, flow cytometry, and fluorescence microscopy. Findings from our study demonstrates the versatility of conjugating efflux substrates with fluorophores to explore efflux systems in Mycobacteria.

81

Computational Chemogenomics-Enabled Drug Discovery Efficiently Identifies Novel Compounds Active Against Mycobacteriodes abscessus

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Abstract

Effective treatments for Mycobacteriodes abscessus infections have been elusive, prompting efforts to repurpose existing anti-infectives, often with disappointing results owing to a high level of innate drug resistance. New strategies exploring a broader chemical space are required, though hit rates in large unbiased screens have been discouragingly low. We have evaluated a novel approach employing computational chemogenomics-enabled drug retargeting that efficiently reveals active compounds paired with small sets of predicted targets. Several of the hits exhibited rapid bactericidal activity, both in axenic culture and infected phagocytes. Interestingly, starvation, which typically induces drug tolerance, sensitized M. abscessus to some of the compounds, resulting in potencies similar to drugs in clinical use. The value of the target predictions that are intrinsic to the method was validated by the suppression of activity of a compound that was predicted to target amino acid biosynthetic pathways by supplementation with Casamino acids. This innovative drug discovery platform has potential to rapidly replenish development pipelines, which is particularly urgent for non-tuberculous mycobacterial infections with limited and poorly effective treatment options.

82

In vitro activity of pravibismane against Mycobacterium abscessus and Mycobacterium avium strains

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Abstract

Nontuberculous mycobacteria (NTM) causes chronic pulmonary disease and is increasingly prevalent. There is a need to develop treatments to limit intracellular NTM infections within human cells. The use of pravibismane, first in a new class of therapeutics that demonstrates broad-spectrum anti-infective and anti-biofilm activity, was investigated to reduce bacterial burden. Mycobacterium abscessus and Mycobacterium avium infections were carried out in THP-1 human alveolar macrophages and A549 human lung epithelial cells. In Mueller Hinton broth culture of M. abscessus 1513, pravibismane exhibited lower minimum inhibitory concentration (MIC) compared to amikacin. The MIC of pravibismane against M. avium strains 2285S and 2285R was lower compared to control rifampin MIC in THP-1 cells. Similarly, pravibismane exhibited slightly lower MIC in M. abscessus strain 1513 infected THP-1 cells relative to amikacin. The efficacy of pravibismane (0.3125 µg/ml - 20 µg/ml) in reducing intracellular M. abscessus or M. avium burden in THP-1 and A549 cells was determined using bacterial colony count method (CFU). Pravibismane at concentrations ≥ 2.5 µg/ml reduced intracellular bacterial burden of M. abscessus strains 21, 1513, and 19977, and M. avium strains 2285R, 2285S, and 101 relative to untreated control in both THP-1 and A549 cells. Flow cytometric analysis demonstrated that ≥ 95% THP-1 and A549 cells remain viable following treatment with 20 µg/ml pravibismane. Our in vitro results indicate that pravibismane results in MIC values and reduction of intracellular bacterial burden that is superior to rifampicin and should be further evaluated in chronic M. abscessus and M. avium mouse models.

83

Understanding antibiotic penetration into mycobacterial biofilms using NanoSIMS

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Abstract

Mycobacterium abscessus is an opportunistic, drug-resistant, nontuberculous mycobacteria (NTM) pathogen associated with chronic pulmonary infections, especially in individuals with cystic fibrosis. Biofilm formation can take place along the alveolar walls of such patients following inhalation of **M. abscessus** from environmental reservoirs. These biofilms have an increased level of antimicrobial resistance (AMR) and are difficult to eradicate. Treatment for **M. abscessus** infections often requires administration of a cocktail of antibiotics for over two years and is frequently unsuccessful. Bedaquiline (BDQ), is an approved antibiotic used for the treatment of multidrug- resistant tuberculosis, which inhibits mycobacterial ATP synthase and evidence has shown **in vitro** efficacy against NTMs. The question being addressed is whether the increased AMR and treatment time in **M. abscessus** infection is due to lack of antibiotic penetration into the biofilm**.** The susceptibility of **M. abscessus** grown as planktonic bacilli and biofilms to the antibiotic bedaquiline (BDQ) was measured as the minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC), respectively. The MBEC of BDQ was 16 times higher (4µg/ml) compared with the MIC (0.25µg/ml). In addition, nano scale secondary ion mass spectrometry (NanoSIMS) was used to assess the penetration of BDQ into **M. abscessus** within biofilms. This was achieved by analysing the ratio of the uptake of Br^- ion in BDQ with the organic elements in the individual cells of **M. abscessus** biofilms. Understanding antibiotic penetration and AMR generation in NTM biofilms could lead to the development of novel treatment strategies.

84

Mycobacterium tuberculosis DprE1 inhibitor OPC-167832 is active against Mycobacterium abscessus

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Abstract

Inhibitors of Mycobacterium tuberculosis decaprenylphosphoryl-β-D-ribose oxidase (DprE1) have emerged as promising candidates for tuberculosis (TB) treatment. A few DprE1 inhibitors have been tested against Mycobacterium abscessus (Mab) and found to be inactive, thus calling into question the 'vulnerability' of the Mab DprE1 homolog (Mab_0192c). As part of our strategy to identify new anti-Mab drugs by screening TB actives, we screened current DprE1 inhibitors and confirmed that most of the compounds lacked anti-Mab activity. Surprisingly, we found the carbostyril derivative OPC-167832 to be active. This clinical drug candidate displayed potent activity against subspecies reference strains and a collection of clinical isolates of Mab. Drug potency interaction studies showed that the compound did not antagonize the activity of commonly used Mab antibiotics, suggesting that OPC-167832 could be co-administered with current drugs. Importantly, OPC-167832 showed efficacy in a Mab mouse infection model. Isolation and sequencing of spontaneous resistant mutants confirmed the Mab DprE1 homolog as the compound's target. This sequencing exercise also revealed mutations in the homolog of RNA polymerase sigma factor SigA (Mab_3009) as a major resistance mechanism in mutants without Mab DprE1 mutations. It is noteworthy that we observed a dramatic potency difference in Middlebrook 7H9 vs cation-adjusted Mueller Hinton (CAMH) broth, which was uncovered to be due to protein binding in the culture medium. In conclusion, we validated the DprE1 homolog as an attractive drug target for Mab and identified OPC-167832 as a novel, oral and bactericidal drug candidate for the treatment of Mab lung disease.

85

Human mesenchymal stromal cells inhibit Mycobacterium avium growth in in vitro and in vivo models of pulmonary infection

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Abstract

Aims

New therapeutic strategies are needed for Mycobacterium avium pulmonary infection. Mesenchymal stromal cells (MSCs) are mature multipotent cells with antimicrobial and immunomodulatory properties. We investigated the therapeutic potential of MSCs using in vitro and in vivo models of M. avium pulmonary infection.

Methods

Human monocyte-derived macrophages (MDMs) were infected with M. avium Chester strain and treated with human bone marrow-derived MSCs. Colony-forming units (CFU) were counted for extracellular and intracellular bacteria after 72 hours.

Balb/c mice were administered aerosolised M. avium and treated with $1x10^6$ intravenous human bone marrow-derived MSCs (or placebo) at 21 days and 28 days post infection (p.i.). Lungs, liver and spleen were harvested at day 42 p.i. for bacterial counts. Cytokines were quantified by ELISA.

Results

MSCs reduced numbers of intracellular bacteria in MDMs over 72 hours (median 40% reduction, IQR 20-50%, $p<0.05$). Extracellular CFU were unaffected by MSCs. MSC treatment of infected MDMs led to increased concentrations PGE2 (median 10.1-fold, $p<0.05$). Blocking MSC PGE2 production by COX2 inhibition led to abrogation of their antimycobacterial effect, which was restored through adding exogenous PGE2.

MSC-treated mice had a median 20% reduction in pulmonary CFU (IQR 10-40%, $p<0.05$). but no change in liver and splenic bacterial counts.

Conclusions

Human MSCs inhibited intracellular survival of M. avium in macrophages and reduced pulmonary counts in a mouse model of chronic NTM infection. Cellular studies suggest MSCs disrupt intracellular M. avium growth in a COX2/PGE2-dependent manner. Further evaluation of MSCs as an adjunctive therapy in anti-mycobacterial treatment is warranted.

86

Shredding Intracellular NTMs with a Macrophage-Targeted Enzymatic Cocktail

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Abstract

To address intracellular mycobacterial infections, Endolytix developed a cocktail of four enzymes that catalytically attack 3 layers of the mycobacterial envelope that is delivered to macrophages through a targeted drug delivery vehicle. This unique combination of enzymes leverages some enzymes encoded by bacteriophages while others come from other organisms thus allowing degradation of the mycobacterial envelope from the outside in, reversing traditional bacteriophage-based approaches while avoiding bacterial surveillance pathways that cleave bacteriophage genomes. Lysin A, a mycobacteriophage encoded protein is thought to cleave the peptidoglycan layer. Lysin B, also mycobacteriophage derived, is an esterase that hydrolyzes the linkage between arabinogalactan and mycolic acid layers. The problem of providing access to the substrates of Lysin A and Lysin B exogenously was addressed by adding enzymes that would degrade the extracellular capsule shown to present in M. tuberculosis and expected to be prevalent in NTMs as determined by host range studies we have conducted so far. We demonstrate our drug formulation is bactericidal in both in vitro and ex vivo experiments. Here we demonstrate a mechanism of action that results in rapid mycobacterial cell death by fragmentation into subcellular fragments. We demonstrate the ability to rescue macrophages from the necrotic cell death of mycobacterial infection.

87

The arylvinylpiperazine amide AX-35 targets the cytochrome bc_1 in Mycobacterium abscessus.

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Abstract

In cystic fibrosis patients, non-tuberculous mycobacteria (NTM) are opportunistic, intracellular pulmonary pathogens that contribute to lung function deterioration and, ultimately, morbidity due to respiratory failure. Of the NTM respiratory pathogens, Mycobacterium abscessus complex (MABSC) poses additional treatment complications as they are notoriously drug-resistant. Despite belonging to the same genus as Mycobacterium tuberculosis (M.tb), MABSC is resistant to most antitubercular drugs, resulting in only a handful of active drugs against these bacteria. As part of our routine screening against other mycobacterial species, one of the lead anti-TB candidates, the cytochrome bc_1 inhibitor AX-35, was found to be active against MABSC isolates in vitro (MIC≤ 15µg/mL) and ex vivo (M. abscessus-infected macrophages). Isolation and characterization of AX-35 resistant mutants (MIC AX-35 ≥ 250µg/mL) revealed the presence of a chimeric gene resulting from the recombination of MAB_2467 in MAB_1966c. In M. abscessus, both genes encode for the b subunit of the cytochrome bc_1 oxidase (QcrB). Further analysis, comprising gene inactivation and ATP-depletion assay, suggested that MAB_1966c is the target of AX-35. Interestingly, transcriptomic analysis of M. abscessus treated with AX-35 revealed that the compound triggered the expression of MAB_2467 and the alternate terminal oxidase, the cytochrome bd. In M.tb, in the absence of the cytochrome bd oxidase, QcrB inhibitors, including AX-35, are bactericidal. However, in M. abscessus, AX-35 had bacteriostatic activity. These results suggested that, in the presence of AX-35, MAB_2467 may compensate for the absence of the cytochrome bd oxidase.

Environmental Aspects of NTM Infections

Poster Session - Saturday, June 4

11:50 am - 1:30 pm

88

Structure of bacterial communities in Japanese-style bathrooms: Comparative sequencing of bacteria in shower water and showerhead biofilms using a portable nanopore sequencer

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Abstract

Showers are one of the main exposure routes to diverse microbes for end users in built environments. Bacteria in water are responsible for biofilm formation on surfaces, and the inside of a showerhead is a specific niche. Here, for the purpose of microbial characterization, source estimation and possibility of infection, the bacterial compositions of both shower water and showerhead biofilms in the same bathrooms were determined and compared using a portable nanopore sequencer. The results suggest that specific bacteria in source water would primarily adhere to the surface of the showerhead where they subsequently form biofilms, and the community compositions within biofilms largely vary depending on environmental factors. The relative abundance of several pathogenic bacterial genera in both water and biofilm samples was low. We suggest that it is important to manage risk of infection in each household, and rapid on-site analysis of microbial communities will allow the realization.

89

Comparing methods for Mycobacterium avium biofilm growth in vitro

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Abstract

Biofilms containing the opportunistic pathogen Mycobacterium avium are likely a source of environmental exposure leading to infections. While a number of methods have been used to generate biofilms of M. avium, it is unknown whether different approaches generate similar structures and cell phenotypes. To make a side-by-side comparison, we chose two published methods for generating M. avium biofilms: by incubating in M63 medium for four weeks or by inducing reductive stress using dithiothreitol (DTT) for 24 hours. Comparison of biofilm ultrastructures using scanning electron microscopy (SEM) revealed differences in biofilms formed by the two methods, especially in the appearance of extracellular material present. We tested the ability of different enzymes to disrupt biofilm integrity in each model, revealing likely differences in extracellular matrix (ECM) structure: the DTT model was heavily degraded by Proteinase K, moderately degraded by Cellulase, and not degraded by DNase. The M63 model was moderately degraded by DNase and Cellulase, but not degraded by Proteinase K. Both models decreased susceptibility to the bactericidal effects of amikacin and clarithromycin, relative to planktonic bacteria. In all cases, 10-1000 fold reductions in killing were observed between biofilm and planktonic cells at the highest drug concentration (512µg/ml). Trends suggest that M63 biofilm bacteria were more resistant to the drugs than DTT biofilm bacteria in some cases. These observations demonstrate differences in structure and cell phenotype in M. avium biofilms formed using different methods, and should help inform the use of in vitro biofilm models for studying M. avium.

90

Comparison of three culture media for the detection of rapid-growing nontuberculous mycobacteria in environmental samples

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Abstract

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment and certain species can cause serious infections. Improved environmental surveillance and testing methods are needed to combat the rise in NTM disease incidence. Recently, two methods were developed to improve NTM detection. The MYChrOme™ Culture Plate (Phigenics, LLC), designed specifically for water samples, is the first chromogenic media for rapid-growing NTM detection (patent-pending). NTM Elite agar (Biomerieux), was developed for rapid-growing NTM detection in clinical samples and was recently utilized for environmental samples. This study compared these diagnostics to Middlebrook 7H11 selective media (7H11S) (ASTM E2563-07 method modified for water). Fifty water samples (25 potable and 25 non-potable) were analyzed in triplicate by each method and statistical analysis was performed on each sample. This study showed that the MYChrOme method was overall equivalent to or better than 7H11S media and NTM Elite agar for detection of rapid-growing NTM in potable water. All three methods detected similar amounts of NTM in the non-potable water samples. The chromogenic property of MYChrOme allowed NTM colonies to be more quickly identified and differentiated from other bacteria in comparison to 7H11S media. NTM Elite agar does not require a decontamination step, it has 85.4% selectivity for NTM, and additional analysis is required for colony conformation. The use of innovative environmental NTM diagnostics, in addition to proper water management, can greatly reduce the risk of NTM disease.

91

Characterization of Mycobacterium porcinum isolated from Hawai'i feral pig, environmental, and respiratory samples

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Abstract

Hawai'i has the highest prevalence of NTM pulmonary disease in the United States. From 2,831 Hawai'i samples representing freshwater biofilms, soil, and dust, 109 (3.8%) yielded viable Mycobacterium porcinum. M. porcinum was the fourth most frequently isolated NTM species in this collection. M. porcinum causes lymphadenitis in pigs, as well as human wound infections and osteomyelitis. Because the Hawaiian Islands is home to large populations of invasive feral pigs, we hypothesized that these pigs are plausible animal reservoirs for NTM. In 2021, matched nasal (n=50) and fecal samples (n=50) were collected from deceased O'ahu feral pigs. Soil samples (n=50) were collected near the dispatch site for each pig. Samples are currently undergoing microbiological culture and genomic characterization for NTM. Meanwhile, a panel of four environmental and two respiratory-derived M. porcinum isolates were assessed for morphotype features, pellicle formation, growth in 7H9 and survival in human THP1 macrophages. Macrophages were infected using a multiplicity of infection of 1:1, and changes in CFU were monitored up to 96hrs post infection. M. porcinum isolates tested showed smooth morphotypes and the inability to form pellicles. Dust-derived M. porcinum showed reduced replication in 7H9 media ($p<0.05$) and enhanced control by THP1 macrophages ($p<0.05$) compared to water-derived/respiratory isolates. Host immune responses to M. porcinum will be monitored by quantifying changes in TNF and IL-1β from cell culture supernatants. While M. porcinum is a rare respiratory pathogen in humans, discovery of animal-derived NTM will facilitate new studies to expand our current limited understanding of M. porcinum virulence.

92

Ash transmission of nontuberculous mycobacteria with the Kīlauea volcanic eruption of 2018

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Abstract

Nontuberculous mycobacteria (NTM) are environmental opportunistic pathogens that cause pulmonary disease in susceptible individuals. NTM infections are emerging in the United States, with Hawai'i showing the highest prevalence rates. Toxic gases and airborne particulates released from the Kīlauea volcano on Hawai'i are respiratory hazards and may cause exacerbation in those with pre-existing lung conditions. We hypothesized that Kīlauea volcanic ash served as a fomite carrier and spread NTM to individuals residing in Hawai'i. To test this hypothesis, we collected one ash sample following the 2018 Kīlauea volcanic eruption to compare against freshwater biofilms and soil from the surrounding region. Our analysis revealed eight respiratory relevant NTM isolates microbiologically recovered from this single ash sample, including Mycobacterium abscessus (MAB; n=5/8), Mycobacterium avium (MAV; n=2/8), and Mycobacterium chimaera (MCHIM; n=1/8). In areas surrounding Kīlauea, 44 sites were sampled and 26 (59.1%) were culture positive for NTM. Among the 59 NTM isolates recovered from water biofilms and soil surrounding Kīlauea, we identified MAB (11/59; 18.6%), MAV (6/59;10.2 %), MCHIM (5/59; 8.5%). Phylogeographic analyses comparing core-genome SNPs and geospatial relationships of the NTM isolates recovered from Kīlauea ash, surrounding water and soil, and respiratory isolates revealed that Kīlauea-derived MAB share high genomic similarity (≤36 SNPs). Matches were observed between three (3/3=100%) Kīlauea ash-derived MAB isolates, 15 (15/26=58%) surrounding environmental MAB isolates, and 6 (6/10=60%) clinical MAB isolates. These findings implicate that volcanic particulates may serve as a previously unrecognized fomite for respiratory transmission of NTM, leading to environmentally-acquired NTM lung disease.

93

Phylogeographic analyses of nontuberculous mycobacteria in Hawai'i households implicates risk of transmission from plumbing sources

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Abstract

Phylogeography combines geographic information with phylogenetics to understand the emergence, spread, and evolution of pathogens. Environmental acquisition of NTM is believed to cause most infections. NTM occupy broad ecological niches including soil, freshwater, single-cell microbes, and plants while causing infection in a range of animals. Hawai'i has the highest prevalence of NTM infection in the United States. To generate epidemiological hypotheses, we phylogeographically compared 309 genomes of Mycobacterium abscessus (MAB; n=124) and Mycobacterium avium complex (MAC; n=185) isolates and their geospatial relationships from 92 sites within Hawai'i. NTM isolates were microbiologically recovered from household water biofilms and soil, in addition to clinical isolates from individuals with NTM in Hawai'i. We identified genomic similarity (i.e., MAB ≤30 SNPs and MAC ≤20 SNPs), indicating NTM transmission between the environment and individuals with NTM. In clinical MAB comparisons, the most frequent matches were to isolates from kitchen sink faucets (39.5% matches), public sink faucets (19.9%) and beach showerheads (17.1%); whereas, the clinical MAC were most frequently matched to isolates from residential showerheads (35.1%), kitchen sink faucets (20.6%), public sink faucets (19%). In four households with both NTM patient and environmental isolates, 50% (2/4) showed genomic matches indicative of recent transmission: one patient's clinical isolate matched a MAB isolate from their kitchen sink and the other patient's clinical isolate matched a M. chimaera from their showerhead. These identified genomic matches underscore the importance of these environmental niches in Hawai'i and can help guide future infection control measures.

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