



ERS | *monograph*

The Lung Microbiome

Edited by Michael J. Cox,
Markus J. Ege and
Erika von Mutius

The Lung Microbiome

Edited by
Michael J. Cox, Markus J. Ege
and Erika von Mutius

Editors in Chief
Robert Bals (outgoing Editor)
John R. Hurst (incoming Editor)

This book is one in a series of *ERS Monographs*. Each individual issue provides a comprehensive overview of one specific clinical area of respiratory health, communicating information about the most advanced techniques and systems required for its investigation. It provides factual and useful scientific detail, drawing on specific case studies and looking into the diagnosis and management of individual patients. Previously published titles in this series are listed at the back of this *Monograph*.

ERS Monographs are available online at www.books.ersjournals.com and print copies are available from www.ersbookshop.com

Editorial Board: Antonio Anzueto (San Antonio, TX, USA), Leif Bjermer (Lund, Sweden), John R. Hurst (London, UK) and Carlos Robalo Cordeiro (Coimbra, Portugal).

Managing Editor: Rachel Gozzard
European Respiratory Society, 442 Glossop Road, Sheffield, S10 2PX, UK
Tel: 44 114 2672860 | E-mail: monograph@ersnet.org

Production and editing: Caroline Ashford-Bentley, Jonathan Hansen, Claire Marchant, Kay Sharpe and Ben Watson

Published by European Respiratory Society ©2019
March 2019

Print ISBN: 978-1-84984-101-6
Online ISBN: 978-1-84984-102-3
Print ISSN: 2312-508X
Online ISSN: 2312-5098

Typesetting by Nova Techset Private Limited
Printed by Page Bros Group Ltd, Norwich, UK

All material is copyright to European Respiratory Society. It may not be reproduced in any way including electronic means without the express permission of the company.

Statements in the volume reflect the views of the authors, and not necessarily those of the European Respiratory Society, editors or publishers.



Contents

The Lung Microbiome	Number 83 March 2019
Preface	v
Guest Editors	vii
Introduction	ix
List of abbreviations	xii
1. Sampling <i>Robert P. Dickson and Michael J. Cox</i>	1
2. Techniques: culture, identification and 16S rRNA gene sequencing <i>Gisli G. Einarsson and Sébastien Boutin</i>	18
3. Applying clinical metagenomics for the detection and characterisation of respiratory infections <i>Themoula Charalampous, Gemma L. Kay and Justin O'Grady</i>	35
4. Applying ecological theories in research: lessons learned from microbial ecology and evolution? <i>Bärbel Ulrike Foessel, Stefan Pfeiffer, Abilash Chakravarthy Durai Raj, Susanne Krauss Etschmann and Michael Schloter</i>	50
5. The early-life microbiome: the key to respiratory health? <i>Emma M. de Koff, Celine Pattaroni, Benjamin J. Marsland and Debby Bogaert</i>	67
6. From culturomics to metagenomics: the mycobiome in chronic respiratory diseases <i>Louise-Eva Vandenberght, Raphaël Enaud, Noémie Coron, David Denning and Laurence Delhaes</i>	88
7. Viruses <i>William G. Flight, Christopher J.R. Turkington and Martha R.J. Clokie</i>	119
8. Obstructive airways disease: potential pathogenetic roles <i>Kian Fan Chung, Gary B. Huffnagle and Yvonne J. Huang</i>	140
9. Chronic suppurative lung disease: cystic fibrosis and non-cystic fibrosis bronchiectasis <i>Geraint Rogers and Yvonne J. Huang</i>	158

10. Interstitial lung disease 173
David N. O'Dwyer, Bethany B. Moore and Philip L. Molyneaux
11. The lung bacterial microbiome in community-acquired and nosocomial pneumonia 188
Lieuwe D.J. Bos, Jamie Rylance and Stephen B. Gordon
12. Compromised immunity: transplantation, cancer and HIV 195
Eric Bernasconi, John-David Aubert, Angela Koutsokera, Laurent P. Nicod and Homer L. Twigg III
13. The environmental microbiota and asthma 216
Pirkka V. Kirjavainen, Heidi Hyytiäinen and Martin Täubel
14. Challenges, impact and the future 240
Michael J. Cox, Markus J. Ege and Erika von Mutius



Preface

Robert Bals (outgoing Editor in Chief) and
John R. Hurst (incoming Editor in Chief)

The concept of microbiome research is a hot topic and multiple diseases have been linked with a dysbiosis at various sites of the body. The lay press has featured many aspects of this topic and attracted attention to this research. The basic techniques of this field have been developed outside the medical area and originate in terrestrial microbiology. The methodologies have developed rapidly and are closely linked with NGS, high-throughput analysis, personalised medicine and bioinformatic analysis of big data. While most papers in this area address the microbiome of the gut, the field of microbiome research in pulmonary medicine has also attracted a lot of activity.



Robert Bals

The microbiome of the lung is a fascinating area because it allows us to integrate a novel view on the coexistence of the body and micro-organisms into mechanisms of disease development. The lung must have a low abundance of micro-organisms and only minor inflammation in order to function adequately. Strong host defence instruments quickly eliminate micro-organisms. Genetic and environmental harm cause a breach in the pulmonary host defence, with subsequent dysbiosis. There are reports that describe alterations of the microbiota in almost all lung disease entities. Nevertheless, it is often unclear whether these changes have a role in disease development or whether they are a secondary result of the underlying disease.



John R. Hurst

This *Monograph* provides the reader with a broad and detailed overview of various aspects of the lung microbiome. The Guest Editors, Michael J. Cox, Markus J. Ege and Erika von Mutius, have brought together chapters that consider methodology and ontogenesis, as well as disease-specific chapters, on asthma, COPD, CF, interstitial lung disease and pneumonia. I thank the Guest Editors for their work; together with the authors of the individual chapters, they have produced a unique and comprehensive introduction to the field. I am sure this book will help develop understanding about the concepts and application of this novel area of research.

This is the last *ERS Monograph* that I, Robert Bals, will handle as Editor in Chief, and I now hand over to the incoming Editor in Chief John R. Hurst. During my time as Editor in Chief, I have tried to focus on topics that are relevant to the physician at the bedside. And I feel that together with the outstanding work of the guest editors, the many authors involved and the ERS Publications Office, we have produced an excellent series of books. It has been a great pleasure and an honour to serve as *ERS Monograph* Editor in Chief – thank you!



Guest Editors

Michael J. Cox

Michael J. Cox is a microbial ecologist and postdoctoral research associate in the Genomic Medicine section of the National Heart and Lung Institute, Imperial College London (London, UK). He trained in the marine microbial ecology of atmospheric trace gases at the University of Warwick (Coventry, UK) and Plymouth Marine Laboratory (Plymouth, UK) in 2005, following this with work on marine polysaccharide degrading bacteria at the University of Liverpool (Liverpool, UK). In 2008, he moved to the University of California San Francisco (San Francisco, CA, USA) as a visiting research fellow on the microbial ecology of CF, returning to the UK and Imperial College London in 2010. Here he has established the microbiome sequencing pipelines at the National Heart and Lung Institute and works on applying microbial ecology techniques to understanding the respiratory microbiome in chronic and acute respiratory diseases. He is a co-director of the recently established Microbiome@Imperial network, an editor at *FEMS Microbiology Letters*, a member of the Society for Applied Microbiology and is active in public engagement, and equality and diversity, establishing www.shenotespeaker.org to promote the visibility of women in respiratory, cardiology, vascular and critical care research.



Markus J. Ege

Markus Ege is a professor of clinical respiratory epidemiology at LMU Munich (Munich, Germany). He trained as paediatrician and epidemiologist at the universities in Ulm (Germany) and Munich. His major research interest is the environmental determinants of childhood asthma and allergies, and their interaction with genetic background. He has worked on asthma and allergy protective effects through bacterial and fungal exposure, with particular focus on the farming environment.



Erika von Mutius



Erika von Mutius is a professor of paediatric allergology and holds a degree in epidemiology from Harvard School of Public Health (Boston, MA, USA). She is head of the Asthma and Allergy Department at the Dr. von Hauner Children's Hospital in Munich (Germany). Since 2017, she has lead the Institute for Asthma and Allergy Prevention at Helmholtz Zentrum Munich – German Research Center for Environmental Health (Neuherberg, Germany). She is also a member of the German Centre for Lung Research (DZL).

Erika von Mutius is a fellow of the European Respiratory Society and was a member of the editorial board of the *New England Journal of Medicine* until the end of 2018. In 2010, she was awarded an honorary doctorate from the University of Helsinki (Helsinki, Finland) and in 2013, she received the Gottfried-Wilhelm-Leibniz Prize of the German Research Foundation, the most important research award in Germany.

Erika von Mutius and her working group have been actively involved in the design, implementation and data analysis of many large Pan-European multicentre and interdisciplinary projects, addressing the potential role of genetic and environmental factors for the development of allergic diseases. The group has furthermore developed expertise and gained excellence in the conduct of studies aimed at elucidating the role of the human and environmental microbiome for the development of allergies and asthma. Erika von Mutius has published widely on different aspects of the disease and is a sought-after speaker at congresses all over the world.



Introduction

Michael J. Cox¹, Markus J. Ege^{2,3} and Erika von Mutius^{2,3,4}

 @ERSpublications

The lung microbiome is an emerging context for respiratory disease. This book: introduces the field; discusses methodology; consolidates knowledge of the influence of microbial communities; considers new developments; and discusses therapeutic use. <http://ow.ly/1oSD30nGD0Z>

In constant contact with the wider environment, the respiratory tract and lungs are continually exposed to micro-organisms. It has been demonstrated, using both culture-independent and -dependent techniques that there are microbial communities in the healthy LRT. The findings have been remarkably consistent and particular organisms are common to this environment and differ between health and disease in both the number and particular types of organism. Different communities of bacteria are present in different diseases, and in different disease states. We are beginning to understand the early development of the lung microbiome, which accrues organisms as infants grow. Selection by the host environment and immune system also play a part, as it is not a random collection of environmental organisms that can be found but a reproducible subset. There are interactions between the lung microbiome, the oral microbiome and the gut microbiome that may significantly shape the microbiome of the lower airways; these interactions are currently being explored.

Respiratory diseases are amongst the most common causes of morbidity and mortality worldwide and whether acute or chronic, micro-organisms are involved in all of them. This can be in susceptibility to disease, in its cause, in inflammation, in response to disease or in response to treatment. Though much progress has been made in the last decade, these processes are not well understood in the context of the microbiome.

Studying the lung microbiome requires specialist approaches to sampling, laboratory techniques and statistical analysis against the background of a rapidly developing field, which tends to focus on more easily studied host environments, such as the gut. In this *Monograph*, we introduce the techniques used and how respiratory sampling [1], 16S rRNA gene sequencing [2], metagenomics [3] and the application of ecological theory [4] can be used to examine the respiratory microbiome experimentally. Following this, we examine different

¹National Heart and Lung Institute, Imperial College London, London, UK. ²Dept of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Munich, Germany. ³Comprehensive Pneumology Center Munich, Member of the German Center for Lung Research, Munich, Germany. ⁴Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Institute for Asthma and Allergy Prevention, Neuherberg, Germany.

Correspondence: Michael J. Cox, Section of Genomic Medicine, National Heart and Lung Institute, Guy Scadding Building, Royal Brompton Campus, London, SW3 6LY, UK. E-mail: michael.cox1@imperial.ac.uk

Copyright ©ERS 2019. Print ISBN: 978-1-84984-101-6. Online ISBN: 978-1-84984-102-3. Print ISSN: 2312-508X. Online ISSN: 2312-5098.

aspects of the respiratory microbiome in a range of different contexts: the paediatric microbiome and how this develops [5]; viruses [6] and fungi [7] as part of the microbiome; in addition to the more frequently studied bacteria. We move on to explore the respiratory microbiome in asthma and COPD [8], chronic suppurative lung diseases [9], interstitial lung diseases [10], acquired pneumonia [11], transplantation, cancer and HIV [12], and the interaction of the respiratory microbiome and the environment [13].

We invite readers to compare and contrast the findings in each area, because although individuality is a feature of host-associated microbial communities, common threads can be found in each. There are now many active researchers in the field, with different insights into the various aspects of the respiratory microbiome, and this is an excellent time to take stock of how it might develop and have an impact on research and clinical approaches to reducing disease.

We thank our authors and our expert reviewers for their work on this *Monograph*, which include amongst them early career and senior authors from many leading groups in the field. Here we have attempted to collate and underline their achievements, highlight those of other researchers, while informing those new to the field about the current state of the art.

References

1. Dickson RP, Cox MJ. Sampling. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 1–17.
2. Einarsson GG, Boutin S. Techniques: culture, identification and 16S rRNA gene sequencing. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 18–34.
3. Charalampous T, Kay GL, O’Grady J. Applying clinical metagenomics for the detection and characterisation of respiratory infections. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 35–49.
4. Foesel BU, Pfeiffer S, Raj ACD, *et al.* Applying ecological theories in research: lessons learned from microbial ecology and evolution? *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 50–66.
5. de Koff EM, Pattaroni C, Marsland BJ, *et al.* The early-life microbiome: the key to respiratory health? *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 67–87.
6. Flight WG, Turkington CJR, Clokie MRJ. Viruses. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 119–139.
7. Vandenborgh L-E, Enaud R, Coron N, *et al.* From culturomics to metagenomics: the mycobiome in chronic respiratory diseases. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 88–118.
8. Chung KF, Huffnagle GB, Huang YJ. Obstructive airways disease: potential pathogenetic roles. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 140–157.
9. Rogers G, Huang YJ. Chronic suppurative lung disease: cystic fibrosis and non-cystic fibrosis bronchiectasis. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 158–172.
10. O’Dwyer DN, Moore BB, Molyneaux PL. Interstitial lung disease. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 173–187.
11. Bos LDJ, Rylance J, Gordon SB. The lung bacterial microbiome in community-acquired and nosocomial pneumonia. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 188–194.
12. Bernasconi E, Aubert J-D, Koutsokera A, *et al.* Compromised immunity: transplantation, cancer and HIV. *In: Cox MJ, Ege MJ, von Mutius E, eds. The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 195–215.

13. Kirjavainen PV, Hyytiäinen H, Täubel M. The environmental microbiota and asthma. *In*: Cox MJ, Ege MJ, von Mutius E, eds. *The Lung Microbiome (ERS Monograph)*. Sheffield, European Respiratory Society, 2019; pp. 216–239.

Disclosures: M.J. Cox was supported by Professor William Cookson and Professor Miriam Moffatt's Joint Senior Investigator Award from the Wellcome Trust. M.J. Ege reports receiving a grant from the German Federal Ministry of Education and Research (BMBF), during the conduct of the study. M.J. Ege has patents issued (EP000002361632B1 with royalties paid, and US000009950017B2) for naturally occurring microbiota for protection from asthma and allergies. E. von Mutius reports receiving the following, outside the submitted work: personal fees from Boehringer Ingelheim, HAL Allergie GmbH and Nestlé Deutschland AG for acting as a speaker; personal fees from Peptinnovate for consultation; and personal fees from OM Pharma SA for working on an advisory board.

List of abbreviations

BAL	bronchoalveolar lavage
CF	cystic fibrosis
COPD	chronic obstructive pulmonary disease
FEV₁	forced expiratory volume in 1 s
Ig	immunoglobulin
IL	interleukin
LRT	lower respiratory tract
LRTI	lower respiratory tract infection
NGS	next-generation sequencing
rRNA	ribosomal RNA
RSV	respiratory syncytial virus
RTI	respiratory tract infection
TLR	Toll-like receptor
URT	upper respiratory tract
URTI	upper respiratory tract infection