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► To cite this version:

Niki D J Ubags, Miguel A Alejandro Alcazar, Suhas G Kallapur, Sylvia Knapp, Sophie Lanone, et al.. Early origins of lung disease: Towards an interdisciplinary approach: Early origins of lung disease. European Respiratory Review, In press, Epub 2020 Sep. inserm-02920087

HAL Id: inserm-02920087

<https://inserm.hal.science/inserm-02920087>

Submitted on 24 Aug 2020

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1 **Early origins of lung disease: Towards an interdisciplinary approach**

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3 Niki D.J. Ubags^{1*}, Miguel A. Alejandro Alcazar^{2,3,4}, Suhas G. Kallapur⁵, Sylvia Knapp^{6,7},
4 Sophie Lanone⁸, Clare M. Lloyd⁹, Rory E. Morty^{10,11}, Céline Pattaroni¹², Niki L. Reynaert¹³,
5 Robbert J. Rottier¹⁴, Hermelijn H. Smits¹⁵, Wouter A.A. de Steenhuijsen Pitters^{16,17}, Deborah
6 H. Strickland¹⁸ Jennifer J.P. Collins^{14*}

7
8 ¹ Faculty of Biology and Medicine, University of Lausanne, Service de Pneumologie, CHUV,
9 Lausanne, Switzerland

10 ² Department of Paediatrics and Adolescent Medicine, Faculty of Medicine and University
11 Hospital Cologne, Translational Experimental Paediatrics, Experimental Pulmonology,
12 University of Cologne, Cologne, Germany

13 ³ Centre of Molecular Medicine Cologne (CMMC), Faculty of Medicine and University
14 Hospital Cologne, University of Cologne, Cologne, Germany

15 ⁴ Institute for Lung Health, University of Giessen and Marburg Lung Centre (UGMLC),
16 Member of the German Centre for Lung Research (DZL), Gießen, Germany.

17 ⁵ Neonatal-Perinatal Medicine, Department of Pediatrics, David Geffen School of Medicine,
18 UCLA, Los Angeles, California, USA

19 ⁶ Department of Medicine I/Research Laboratory of Infection Biology, Medical University of
20 Vienna, Vienna, Austria

21 ⁷ CeMM, Research Centre for Molecular Medicine of the Austrian Academy of Sciences,
22 Vienna, Austria

23 ⁸ Univ Paris Est Creteil, INSERM, IMRB, F-94010 Creteil, France

24 ⁹ Inflammation, Repair & Development, National Heart & Lung Institute, Imperial College
25 London, London, UK

26 ¹⁰ Department of Lung Development and Remodelling, Max Planck Institute for Heart and
27 Lung Research, Bad Nauheim, Germany.

28 ¹¹ Department of Internal Medicine (Pulmonology), University of Giessen and Marburg Lung
29 Centre, Member of the German Centre for Lung Research, Giessen, Germany

30 ¹² Department of Immunology and Pathology, Monash University, Melbourne, VIC, Australia

31 ¹³ Department of Respiratory Medicine and School of Nutrition and Translational Research in
32 Metabolism, Maastricht University Medical Centre, Maastricht, the Netherlands

33 ¹⁴ Department of Paediatric Surgery, Sophia Children's Hospital, Erasmus University Medical
34 Centre, Rotterdam, The Netherlands

35 ¹⁵ Department of Parasitology, Leiden University Medical Centre, Leiden, The Netherlands

36 ¹⁶ Department of Paediatric Immunology and Infectious Diseases, Wilhelmina Children's
37 Hospital/University Medical Centre Utrecht, Utrecht, The Netherlands.

38 ¹⁷ National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

39 ¹⁸ Telethon Kids Institute, University of Western Australia, Nedlands, Western Australia,
40 Australia

41

42 *Authors are listed alphabetically except for Niki D. Ubags and Jennifer J.P. Collins*

43

44 * Corresponding authors: Jennifer J.P. Collins PhD
45 Department of Paediatric Surgery
46 Sophia Children's Hospital
47 Erasmus MC
48 PO box 2040
49 3000 CA Rotterdam
50 The Netherlands
51 Email: j.dewolf-collins@erasmusmc.nl

52

53 Niki D. Ubags PhD
54 Faculty of Biology and Medicine
55 University of Lausanne

56 Service de Pneumologie
57 CHUV
58 CLE D02-207
59 Chemin des Boveresses 155
60 1066 Epalinges
61 Switzerland
62 Email: Niki.Ubags@chuv.ch

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64 **Running title:** Early origins of lung disease

65
66 **Total word count:** 5580 words, 129 references, 2 figures

67
68 **Key words:** Early life, lung development, BPD, immune maturation, chronic lung disease,
69 microbiome.

70
71 **Take home message:**

72 Future research into early origins of lung disease should be centred around four major focus
73 areas: 1) *policy and education*, 2) *clinical assessment*, 3) *basic and translational research*,
74 and 4) *infrastructure and tools*, and discuss future directions for advancement.

75
76

77 **Abstract**

78 The prenatal and perinatal environment can have profound effects on development of
79 chronic inflammatory diseases. However, mechanistic insight into how the early life
80 microenvironment can impact upon development of the lung and immune system, and
81 consequent initiation and progression of respiratory diseases is still emerging. Recent
82 studies investigating the developmental origins of lung diseases have started to delineate
83 the effects of early life changes in the lung, environmental exposures and immune
84 maturation on development of childhood and adult lung diseases. While the influencing
85 factors have been described and studied in mostly animal models, it remains challenging to
86 pinpoint exactly which factors and at which time point are detrimental in lung development
87 leading to respiratory disease later in life. To advance our understanding of early origins of
88 chronic lung disease and to allow for proper dissemination and application of this knowledge,
89 we propose four major focus areas: 1) policy and education, 2) clinical assessment, 3) basic
90 and translational research, and 4) infrastructure and tools, and discuss future directions for
91 advancement. This review is a follow-up of the discussions at the ERS Research Seminar
92 “Early origins of lung disease: Towards an interdisciplinary approach” (Lisbon, November
93 2019).

94 **Introduction**

95 Chronic respiratory diseases, such as asthma and chronic obstructive pulmonary
96 disease (COPD), place an enormous burden on society. A better understanding of how early
97 life exposures may predispose people to develop chronic respiratory disease is key to
98 developing prevention and treatment strategies. The prenatal and perinatal environments can
99 have profound effects on the development and progression of chronic inflammatory
100 diseases. However, mechanistic insight into how the early-life microenvironment can impact
101 the development of the lung and immune system, and the consequent initiation and
102 progression of respiratory diseases, is still emerging. Recent studies investigating the
103 developmental origins of lung diseases have started to delineate the effects of early-life
104 changes in lung development (1), environmental exposures (2) and immune maturation (3)
105 on the development of childhood and adult respiratory diseases. Alterations in what is
106 considered “healthy” lung development may prime the neonate for increased susceptibility to
107 develop respiratory complications in later life. Although this viewpoint is widely accepted, the
108 exact mechanisms underlying and timeframe in which these alterations take place remain to
109 be unravelled (**Figure 1**).

110

111 **Pre- and perinatal lung development**

112 *Premature delivery interrupts normal lung development*

113 The development of the lung architecture results from the coordinated activity of
114 several cell-driven mechanisms of lung development, together with physical forces such as
115 those that result from breathing movements [1]. These cellular mechanisms include, for
116 example, extracellular matrix (ECM) deposition and remodelling, primarily by myofibroblasts
117 in the developing septa; coordination of epithelial-mesenchymal interactions by growth
118 factors; cell-ECM interactions; and the proliferation, migration, apoptosis, and trans-
119 differentiation of the constituent cells of the developing lung at the correct time and place
120 during lung maturation [2]. Any disturbances to these pathways result in an aberrant lung
121 structure, which in turn, compromises lung gas-exchange function, and contributes to the

122 development of lung disease. One major disturbance is preterm delivery, which accounts for
123 6-12% of the 5.9 million live births annually in Europe [3]. Prematurity is a leading cause of
124 perinatal and childhood mortality [4]. With intensive care in countries with a more advanced
125 health care system, many preterm infants survive but a major complication is
126 bronchopulmonary dysplasia (BPD). Stunted alveolar lung development has emerged as the
127 histopathological hallmark of BPD, which arises from accelerated lung maturation (alveolar
128 epithelial type II cell maturation, surfactant production and alveolar wall thinning) following
129 exposure to corticosteroids and/or (intrauterine) inflammation during prematurity. This is
130 further complicated by the deleterious effects of prenatal and postnatal exposures to
131 inflammatory, oxidant, stretch, and other injurious agents [5, 6]. The consequences of
132 aberrant lung development are evident in adolescent and adult survivors of BPD, who exhibit
133 disturbances in breathing mechanics [7], as well as predisposition to lung disease [8-11].
134 Recent preclinical studies in experimental animal models of BPD support these conclusions,
135 where neonatal exposure to hyperoxia resulted in persistent disturbances to pulmonary
136 vascular function and right-heart remodelling [12, 13], long term effects on airway
137 hyperreactivity [14-16] and increased sensitivity to bleomycin-induced lung fibrosis in adult
138 mice [17]. Thus, a solid body of evidence suggests that injury to the lung in the immediate
139 postnatal period predisposes affected lungs to disease in later life.

140 A relatively common pregnancy complication associated with preterm labour and
141 BPD is acute chorioamnionitis, which is characterized by neutrophilic infiltration and
142 inflammation at the maternal-foetal interface [18]. A surprising consequence of
143 chorioamnionitis is increased production of surfactant in the foetal lung [19]. However,
144 despite the associated increased lung compliance, chorioamnionitis inhibits alveolar and
145 pulmonary vascular development causing changes similar to BPD [20]. These include
146 decreased and aberrant expression of elastin that identifies sites of secondary septation
147 demonstrated in animal models of chorioamnionitis [21, 22]. Although the mechanisms are
148 not entirely clear, NF- κ B activation in innate immune cells and subsequent IL-1 signalling
149 [23-25] inhibit *FGF10* expression in mesenchymal cells. Inflammatory mediators induce

150 TGFβ1 and CTGF signalling and decrease *Shh* mRNA levels and Gli1 protein expression in
151 the distal lung [21, 26, 27].

152

153 **Early life exposures and susceptibility to lung disease**

154 *Maternal exposures and transplacental immune modulation*

155 The maternal immune system plays a fundamental role in protecting the in utero
156 environment [28, 29] and additionally can provide signals that influence developmental
157 trajectories within the foetal immune system, shaping immune functions post birth [30-33].
158 Although mechanisms remain poorly defined, maternal inflammation can promote foetal
159 programming that manifests as enhanced susceptibility to development of asthma (and
160 related diseases) [31, 33, 34]. A broad spectrum of environmental maternal exposures have
161 been implicated including diet/stress/environmental toxins/pathogens, and pre-existing
162 clinical syndromes including obesity and asthma [34-37]. Notably, maternal inflammatory
163 responses can be exaggerated during pregnancy (such as asthma [36, 37] and Influenza
164 [38]). The mechanisms involved in transplacental transmission of maternal-derived
165 inflammatory immune signals are not well understood, but likely involve direct transfer and
166 cell receptor mediated signaling [32]. Of note, foetal stem cells responsive to these
167 programming signals contribute to development of a range of cellular subtypes that interact
168 to play crucial roles in lung homeostatic processes and inflammation, including local lung
169 tissue resident immune cells (e.g. alveolar macrophages and ILC2) and HSC in bone
170 marrow that seed myeloid populations within respiratory tissues, providing multiple pathways
171 for dysregulated immune function.

172 The capacity of the foetal immune system to be trained via maternal environmental
173 signals provides exciting possibilities for prevention of lung diseases after birth. A seminal
174 example is the profound reduction in allergic asthma (and related diseases) in children born
175 to mothers exposed during pregnancy to benign microbial products from traditional farming
176 environments [39-41]. Mechanistic studies using cord blood mononuclear cells (CBMC) from
177 farmers children indicate that protection is associated with accelerated development of post-

178 natal immunocompetence [42, 43]. These findings have been replicated in experimental
179 animal models via exposure of pregnant mice to a variety of microbial extracts, and these
180 processes were found to be TLR-dependent [44-46]. The precise mechanisms that induce
181 transplacental protection against disease are not well characterised, but recent evidence
182 points to innate immune training via metabolic programming within the foetal bone marrow
183 dendritic precursor populations [46-48]. Furthermore, exposure to microbial extracts during
184 pregnancy may also provide potential benefits to support maternal immunity, as recently
185 shown in preclinical animal models [49]. Harnessing the potential of immune training
186 presents exciting opportunities to enhance control/regulation of maternal inflammatory
187 responses for protection of pregnancy, foetal growth and development, in addition to training
188 the foetal immune system for enhanced immunocompetence in the postnatal environment.

189

190 *Neonatal and infant airway microbiota composition and development*

191 Little is known about the crosstalk between immune cells and microbes (bacteria,
192 viruses and fungi) in newborns, but evidence from mouse models suggests that immune
193 priming in the lungs is essential for protection against infections and appropriate responses
194 upon allergen exposure [33]. A study using lower airways samples (tracheal aspirates) from
195 children in the first year of life identified signs of bacterial colonization as early as one day
196 after birth [50]. The authors showed that the lower airways microbiome developed within the
197 first 2 postnatal months. During this period, three microbiome profiles were evident: two were
198 dominated by either *Staphylococcus* or *Ureaplasma*, whereas the third was enriched with
199 diverse bacterial genera, such as *Streptococcus*, *Prevotella*, *Porphyromonas*,
200 and *Veillonella*, all known to be constituents of a healthy adult lung microbiome. Gestational
201 age and delivery mode were important drivers of microbiome composition. The *Ureaplasma*
202 profile was associated with preterm vaginal delivery, whereas the *Staphylococcus* profile
203 correlated with preterm C-sections. The diverse microbiome profile was associated with term
204 birth. Concomitant evaluation of the host gene expression revealed increased expression of
205 IL-33 and genes linked with IgA production pathway with increasing gestational age. This

206 correlated with an increased predicted immunoglobulin A1 protease function of the microbes.
207 This concordance is suggestive of tightly regulated host-immune crosstalk, which may
208 influence the development of the immune system and inform early-life interventions to
209 prevent respiratory diseases.

210 In line with the lower respiratory tract (LRT) microbiota, a birth cohort in 112 healthy
211 infants indicated rapid development of the nasopharyngeal microbiota, characterised by
212 early *Staphylococcus*-dominance, followed by enrichment of *Corynebacterium/*
213 *Dolosigranulum* and a late introduction and predominance of *Moraxella* by the age of 3
214 months [51]. This microbial developmental trajectory is governed by birth mode, feeding
215 type, antibiotics and crowding conditions, among others, and appears to be related to both
216 bacterial and viral respiratory tract infection (RTI) susceptibility. Infants who suffered from
217 more RTIs over the first year of life demonstrated reduced *Corynebacterium/*
218 *Dolosigranulum*-colonization accompanied by an early *Moraxella* introduction already at 1
219 month of age. These findings suggest a ‘window-of-opportunity’, within which timely
220 microbial cues may modulate host immunity and determine RTI susceptibility [52]. Given the
221 theory that lower RTIs may develop following micro-aspiration of upper respiratory tract
222 (URT) pathogens, it was postulated that oral microbiota would show a similar pattern of
223 maturation and association with RTI susceptibility as the nasopharyngeal microbiota.
224 Contrastingly, the oral microbiota assemble even more rapidly into highly stable and niche-
225 specific microbial communities, showing no differences in maturation patterns related to RTI
226 susceptibility. Instead, it was found that niche differentiation within the URT (nasopharynx
227 *versus* oral cavity) is less explicit in children developing more RTIs over the first year of life.
228 This phenomenon was even more pronounced shortly prior to RTI episodes, and
229 characterised by an influx of oral bacteria like *Fusobacterium*, *Prevotella*, *Neisseria* and
230 streptococci into the nasopharynx [53]. These findings indicate that a ‘collapse’ of bacterial
231 community structure may precede RTI symptoms. Moreover, outgrowth of the number one
232 cause of bacterial RTIs, *S. pneumoniae*, is controlled by the local respiratory microbiota,
233 contributing to mucosal homeostasis [54].

234 However, the respiratory microbiota appears to not only be involved in protection
235 against infections, but likely also play a role in controlling the severity of acute infections. In a
236 recent study, particularly respiratory syncytial virus (RSV), *H. influenzae* and *S. pneumoniae*
237 were indicative of infection, regardless of phenotype (pneumonia, bronchiolitis or mixed),
238 suggesting a joint contribution of viruses and bacteria as driver of these infections, whereas
239 the absence of protective commensals was associated with severity [55]. Interestingly,
240 children born preterm are affected at a higher rate by respiratory viruses, including RSV [56,
241 57]. The risk for readmission due to respiratory infections during the first year of life was
242 shown to be 3.6 times higher in infants born in moderate or late preterm stages [57].

243

244 **Cellular mechanisms involved in priming of the developing lung for disease**

245 *Influence of vascular changes on lung development*

246 The vasculature is important for branching morphogenesis of the future airways [58-
247 61], and the pulmonary vessels are an integral part of the systemic circulation, and mainly
248 expand through angiogenesis [62]. Angiogenesis is the process of the formation of new
249 vessels through the expansion and sprouting of existing vessels. These new extended
250 endothelial tubes are highly unstable and require perivascular cells, pericytes, to become
251 fully functional vessels. The interaction between endothelial cells and pericytes is mediated
252 through platelet-derived growth factor (PDGF) β signalling, which induces stabilization of
253 endothelial cells and subsequent differentiation of pericytes into smooth muscle-like cells
254 [63, 64].

255 Smooth muscle precursor cells (perivascular cells) are primed cells that express
256 Kruppel Like Factor (KLF) 4. These cells respond to induced damage in adult mice, such as
257 hyperoxia, by proliferation and migration, which eventually leading to pulmonary
258 hypertension-like vascular abnormalities [65]. By studying paediatric congenital lung
259 abnormalities, such as congenital diaphragmatic hernia (CDH), which is characterized by
260 concomitant vascular problems (persistent pulmonary hypertension), it has become clear
261 that vascular abnormalities associated with CDH already develop in utero. Histological

262 studies in premature and term CDH patients uncovered a thickening of the vascular smooth
263 muscle layer in the mid-sized vessels as well as neo-muscularization of capillaries.
264 Additionally, smooth muscle cells expressed markers associated with a contractile
265 phenotype [66]. Given the role of perivascular cells during angiogenesis, subsequent studies
266 focused on these cells as a potential source of the vascular abnormalities observed in CDH.
267 Clear vascular abnormalities already become apparent at the pseudoglandular and
268 canalicular stage of development in a mouse model for CDH [67], including significant
269 reductions in vessel length and number of branches from the main pulmonary vessels.
270 Moreover, perivascular cells associated with vessels extended more distally compared to
271 controls, indicative of a higher coverage of vessels by these cells, which was also shown for
272 pulmonary hypertension in adults [68]. The perivascular cells in CDH subsequently
273 expressed markers of advanced differentiation, as compared to controls.

274 Upon differentiation, perivascular cells lose the ability to support endothelial cells that
275 form new branches during angiogenesis. As a result, lung development in CDH is
276 characterized by a simplified microvascular development and subsequent hypoplasia, similar
277 to BPD. In both diseases retinoic acid (RA) signalling has been identified as a key pathway.
278 Retinoic acid is required during the formation of the prospective lung field at the onset of
279 lung development, and reduced RA signalling leads to increased TGF β [69], which induces
280 differentiation of pericytes [70]. Moreover, inhibiting RA signalling impaired vessel formation
281 *in vitro* and caused a reduction in the production of collagen IV, which perivascular cells start
282 to secrete upon interaction with endothelial cells, and has been observed in lungs of human
283 CDH patients [67]. Considering the aberrant function of perivascular and endothelial cells in
284 CDH and BPD patients, it will be important to create a better understanding of whether these
285 patients are predisposed to chronic lung and vascular disease and perhaps even infectious
286 and inflammatory disease in later life.

287

288 *Mesenchymal cells in lung alveolar development and injury*

289 The cellular mechanisms underlying the long-term effects of lung disease in infancy
290 have not been clarified. However, recent studies have identified disease-relevant changes in
291 an emerging spectrum of mesenchymal cells, namely fibroblast subsets, as well as in
292 resident immune cells, particularly macrophages, as new cellular mechanisms on priming
293 the developing lung for disease. Mesenchymal cells are important in orchestrating early lung
294 development, by interacting with epithelial and endothelial cells, communicating back and
295 forth to shape the branching structure that becomes the lung [2]. In saccular and alveolar
296 lung development studying mesenchymal cells and their interplay with their niche has long
297 been based on histological and *in vitro* studies. The mesenchymal compartment of the distal
298 lung was long thought to consist of two types of fibroblasts based on structural properties:
299 lipofibroblasts and myofibroblasts [71]. By studying lung regeneration after pneumonectomy
300 in adult mice, it became clear that some lipofibroblasts function not only as progenitor cells
301 for myofibroblasts, but also as orchestrators of new alveolar growth [72]. The notion that
302 stromal stem cells can function as regulators of repair and regeneration, communicating with
303 a variety of epithelial, endothelial, immune and even neuronal cell types, has been
304 extensively studied in amphibians [73, 74]. The discovery of the potent regenerative and
305 anti-inflammatory properties of bone marrow- and umbilical cord derived mesenchymal
306 stromal cells (MSC) has led to a surge in preclinical and clinical studies showing the promise
307 that these cells hold as a therapeutic agent [75]. In BPD, exogenous MSCs (derived from
308 bone marrow or umbilical cord) have the potential to prevent and repair lung injury and
309 perturbed alveolar development [76-78] and are currently the subject of a number of ongoing
310 clinical trials worldwide (Clinicaltrials.gov identifiers NCT04062136, NCT03873506,
311 NCT03558334, NCT03645525, NCT03378063, NCT03631420, NCT03392467,
312 NCT03601416, NCT04255147, NCT02443961). This, however, also leaves us to question
313 ~~of~~ why resident mesenchymal cell types are unable to fulfil this same role and highlights how
314 little we really understand about the dynamics of mesenchymal cells with the developing
315 alveolar niche. Using tracheal aspirates of preterm infants, it was found that the shedding of
316 mesenchymal cells into the airway compartment predicted the development of BPD [79].

317 Moreover, mesenchymal cells derived from the lungs of BPD patients have a strong
318 profibrotic phenotype [79] and lower expression of PDGFR α and β [80], similar to
319 histological findings in the lungs of patients dying with BPD, suggesting that mesenchymal
320 cells potentially contribute to BPD pathogenesis. This hypothesis is further supported by
321 studies reporting that CD146+ lung MSCs exposed to hyperoxia *in vivo* [81] or *in vitro* [82]
322 exhibited a diminished microvascular supportive capacity and an impaired secretion of
323 factors that are important in orchestrating lung development. It has been suggested that
324 exogenous MSC therapy restores the function of perturbed lung MSCs, but future studies
325 will need to show whether this is indeed the case [83].

326 Mesenchymal cells are heterogeneous, making it hard to characterize and delineate
327 mesenchymal cell interaction with the alveolar microenvironment. Lineage tracing studies
328 and more recently single cell RNA sequencing (scRNAseq) have allowed the identification of
329 a variety of different mesenchymal subsets [84-88]. These mesenchymal subsets appear to
330 be not only very heterogeneous but also plastic in phenotype, as different subsets are found
331 at different ages and after various exposures [88-90]. Defining how these expression-based
332 subsets differ functionally, and what role they play in the cellular crosstalk during
333 development, repair and susceptibility for chronic lung disease is a major challenge that
334 remains to be addressed.

335

336 *Macrophages in lung alveolarization*

337 Resident macrophages migrate into the lung in multiple waves during development,
338 and have been hypothesized to contribute to lung development [26, 91]. Upon exposure to
339 inflammation, foetal pulmonary macrophages play an active role in disrupting lung
340 development by actively inhibiting expression of critical genes that are required for
341 developmental processes [23]. While inflammation *per se* has long been considered a key
342 pathological feature of BPD [92], a variety of inflammatory cell types have recently been
343 implicated in perturbations to lung function, and the development of the lung structure
344 associated with BPD. CD11b⁺ mononuclear cells have been demonstrated (using diphtheria-

345 toxin-mediated depletion) to mitigate hyperoxia-induced lung injury in newborn mice [93].
346 These studies were expanded to reveal that an imbalance in Ly6C^{hi}/Ly6C^{lo} monocyte
347 populations mediated hyperoxia-induced lung injury in the developing lung, which could be
348 rescued by interferon- γ treatment [94].

349 Beyond these monocyte populations, alveolar macrophages (AM) were documented
350 to mediate the deleterious impact of hyperoxia on lung alveolarization in newborn mice
351 (using *Csf1r*-lineage depletion) [95], in a study where neutrophils were documented to be
352 without any effect (through antibody-mediated neutrophil depletion studies with anti-Ly6G
353 IgG). These reports highlight the possibility that oxygen toxicity may reprogram immune cells
354 in hyperoxia-exposed developing lungs. These studies are important, since early life
355 exposure of mouse pups to hyperoxia profoundly impacted the adult response to influenza
356 virus infection [96]. This suggests a persistent reprogramming of the inflammatory response
357 during early life, which was attributed to intrinsic changes in hematopoietic cells, and
358 changes in the reparative versus cytotoxic nature of natural killer cells [97]. The mechanisms
359 underlying these persistent changes in immune cell behaviour that are maintained from
360 infancy to adulthood have not been clarified. However, eosinophil-associated RNase 1
361 (Ear1) in type II alveolar epithelial cells was suggested to play a role in this phenomenon
362 [98]. There is clearly tremendous scope for the further exploration of terminal inflammatory
363 cell programming in the lung in the immediate postnatal period, with consequences for both
364 lung development and innate (and perhaps adaptive) immunity that last into adulthood. This
365 is currently considered a priority area for investigational studies [99], and is explored further
366 in the following section, "Early life immune maturation and development of lung disease",
367 below.

368

369 **Early life immune maturation and development of lung disease**

370 *Early life development of the pulmonary immune system*

371 The perinatal/postnatal period represents a critical window in the pulmonary immune
372 development with enduring effects on pulmonary physiology, susceptibility to lung disease

373 and resistance to infections. Postnatal lung developmental changes, genetic influences and
374 novel (micro)environmental cues shape the migration, expansion and/or maturation of
375 pulmonary immune cells. Macrophage precursors, mainly originating from the foetal liver,
376 seed the lungs during embryogenesis [100, 101]. These macrophage precursors at first
377 reside in the interstitial space, to migrate to the alveolar space after birth, where they
378 undergo final differentiation and remain with the potential of local self-renewal [102]. In line
379 with the substantial adaptations of lung tissue at birth, several groups discovered the sudden
380 release of the alarmin IL33 by alveolar epithelial cells type 2 (AEC2) [103, 104]. By studying
381 the impact of IL33 on the postnatal immune development, it became clear that this phase is
382 accompanied by an IL33-driven wave of type 2 immune cells, most prominently shown by
383 the expansion and activation of innate lymphoid cells type (ILC) 2 and eosinophils [103,
384 104]. The expansion of ILC2s shapes the functional performance of newly differentiating AM,
385 by dampening their pro-inflammatory phenotype, at the cost of an increased susceptibility to
386 bacterial pneumonia [103], and at the same time, skews scarcely present neonatal dendritic
387 cells (DC) to drive Th2 responses, thereby promoting asthma development at young age
388 [104]. A scRNAseq study on mouse lungs before and after birth confirmed macrophage
389 developmental trajectories and the IL33-driven ILC expansion [105]. Further, examining the
390 receptor-ligand interaction in lungs, a resident basophil population was revealed, which
391 functions as an amplifier of the IL13 and CSF2 driven maturation and expansion program of
392 AMs after birth [105]. Another cell type shown to exert protective effects against bacterial
393 pneumonia in neonates, are pulmonary ILC3s, which develop postnatally upon the support
394 of lung fibroblast derived insulin-like growth factor 1 (IGF)1 [106]. Importantly so, newborns
395 with BPD exhibit reduced IGF1 levels and ILC3 numbers in their bronchoalveolar lavage
396 fluid, as do neonatal mice with experimental BPD.

397

398 *Allergic immunity*

399 The relatively limited exposure to antigen in utero dictates that newborns are more
400 reliant on innate immune pathways for protection against infections. However, the foetal

401 immune system contains mature T and B cells that are actively suppressed by regulatory T
402 cells [107]. Moreover, nasal associated lymphoid tissue (NALT) is established before birth,
403 while bronchus associated lymphoid tissue (BALT) expands rapidly postnatally [107]. This
404 immature immune system is shaped following postnatal exposure to pathogens exemplified
405 by (bacteria, viruses and fungi), and inhaled particles such as dust, pollen and animal
406 dander. Moreover, genetic makeup is also an important factor in shaping of the immune
407 responses in early life and can consequently influence lung disease development, as
408 evidenced by the high heritability of an allergic phenotype (and asthma). The timing and
409 nature of the exposures has a significant impact on the developing immune system and may
410 result in skewing towards health or disease. The majority of studies to date have used
411 peripheral blood, and have shown that T helper 2 (Th2) cell preference is required for a
412 healthy pregnancy [108], but also that this preference is maintained during the neonatal
413 period, reducing gradually during the first 2 years [109]. However, a deviation from this
414 physiological Th2 skewing, with exaggerated Th2 responses in either pregnancy or the first 3
415 months of life has been associated with an increased risk of subsequent childhood asthma
416 or wheeze [110]. Mouse models have shown that these Th2 cells are critical in development
417 of allergic inflammation [111]. Factors that may result in accentuated or prolonged Th2
418 skewing include maternal allergy [112], but environmental exposures are also critical as has
419 become apparent from the farming exposure studies [113]. Similarly, diet has a profound
420 influence on the systemic microbiome, which impacts upon the developing immune system
421 and thus the trajectory towards disease or health [114]. Although it is apparent that the
422 composition of inhaled exposures have a direct impact on the airway immune profile,
423 mechanistic studies in the context of a developing immune system are scarce, while studies
424 in children predominantly used peripheral blood rather than cells directly isolated from
425 mucosal surfaces.

426 It is apparent therefore, that early life represents a window of both vulnerability and
427 opportunity that impact immune and tissue homeostasis, but it is not known whether
428 pathology progresses because of an imbalance in regulatory cells versus effector cells.

429 Peripheral blood isolated during the first postnatal year shows that the proportion of both
430 resting naïve T regulatory cells (rTreg; CD4⁺CD45RA⁺FoxP3⁺) and activated Treg (aTreg,
431 CD4⁺CD45RA⁻FoxP3^{high}) increased markedly from birth to 6 months of age [115]. In contrast
432 little is known regarding the phenotypes of cells within airways of neonates, although
433 children who develop allergic disease in the first year of life have deficient Treg responses to
434 microbial stimuli, but not allergens, from birth [116], highlighting the importance of skewed
435 immunity in early life.

436

437 *Inter-organ crosstalk*

438 In recent years, the importance of inter-organ crosstalk and interactions in the
439 initiation, development and progression of respiratory disease has become apparent, and
440 the microbiome plays a central role in these processes [117, 118]. Alterations in the gut
441 microbiome composition can influence early life and adult immune development, and
442 consequently affect the development of respiratory disease (gut-lung axis) [119-121].
443 Moreover, antibiotic use is strongly associated with the development of asthma, particularly
444 when antibiotics were taken during the first 6 months of life [122]. In line with these
445 observations, antibiotic treatment in the perinatal period enhances susceptibility to and
446 severity of murine allergic airway inflammation [119, 123]. This effect was suggested to be
447 linked to the loss of Tregs in the colon, which could, likely mediated by the gut-lung axis,
448 enhance the development of allergic airway inflammation. Dietary intake, in particular high
449 fibre diet consumption, alters gut microbiome composition and consequently increases local
450 and circulating short-chain fatty acid (SCFA) levels [34, 124]. Maternal high fibre diet
451 consumption and consequent reduction in allergic airway inflammation in offspring was
452 found to be mediated by maternal-foetal transfer of SCFAs leading to altered gene
453 expression in the foetal lung and changes in immune regulation [34].

454 Recent evidence has indicated that microbial- and age-mediated immune maturation
455 in the skin can determine the nature and severity of allergic skin inflammation using a
456 neonatal skin sensitization model [125]. Interestingly, the nature of the observed skin

457 inflammation (Th2 mediated in neonates and Th2/Th17 mediated in adults) determined the
458 inflammatory profile (eosinophilic vs neutrophilic) in the lung following allergen challenge,
459 suggestive of skin-lung interactions. Moreover, there is now first evidence of inflammatory
460 immune cell seeding in remote non-allergen exposed mucosal tissues [126]. These data
461 indicate that allergen challenge on the skin, in the gut or in the airway induces not only a
462 local allergic inflammatory response, characterized by local eosinophil influx, but can also
463 initiate increased eosinophil frequencies in distant non-allergen exposed mucosal tissues.
464 There is strong evidence of interactions between the skin, gut and the lung. However, future
465 mechanistic studies focusing on this inter-organ crosstalk are needed to dissect the
466 mechanisms underlying these responses and their implications for disease development in
467 early life [127].

468

469 **Future perspectives**

470 Lifelong respiratory health starts before birth. If we can provide each child with a
471 healthy start, the chances of attaining maximal lung capacity in adult life are markedly
472 increased. To advance our understanding of the early origins of lung disease and to allow for
473 proper dissemination and implication of this knowledge, we propose four major focus areas
474 and future directions for advancement within these areas (**Figure 2**).

475

476 *Policy*

477 The largest impact of and earliest time for prevention of lung diseases is prior to birth.
478 Education of mothers, especially by midwives, with respect to exposures to avoid prior to
479 and during pregnancy can be complemented with advice on healthy behaviour. Such advice
480 will require targeted evidence-based information. Policy makers need to be provided with
481 scientific evidence of specific risk factors associated with explicit diseases. This research
482 and policy advice should be an ongoing and active effort as exposure to risk factors might
483 fluctuate and change over time and display regional differences. For interventions and/or

484 protective measures that will prevent disease development, scientific evidence is ideally
485 delivered by randomized-controlled trials

486 In underdeveloped and low-income countries, prenatal disease prevention is yet to
487 become part of public health policies and more effort should be put into raising awareness.
488 In these settings, cascading teaching methods and getting individuals with a respected
489 position in society involved is imperative to spread good practices (*education*). It can be
490 expected that if mothers adopt healthier lifestyles during pregnancy, they will raise their
491 children under healthier circumstances, and this next generation will therefore take this as
492 normal behaviour. This will not only affect early life, but also impact upon the development of
493 adult-onset diseases. Moreover, given the lack of identified biomarkers so far, the focus
494 should be on higher risk groups, with a prominent role for midwives.

495

496 *Clinical assessment*

497 The worst-case scenario of a difficult start to life with respect to lung function is the
498 development of BPD, which we will use here as a prototypic early life lung disease. As
499 survival of preterm infants at lower gestational ages has increased due to improvements in
500 care and treatment, BPD has taken on new dimensions. There is a widespread consensus
501 that the current definition is clinically useful, but too broad [128]. The fact that BPD is not a
502 single disease, but rather a syndrome, is not captured by this definition. Moreover, the
503 pathological features underlying the disease are difficult to assess in the clinic. In addition,
504 the long-term consequences of BPD, such as airflow limitation, need to be clearly defined
505 and the question remains whether this should be termed as an early COPD phenotype.
506 Better imaging techniques, including Magnetic Resonance Imaging, are needed to obtain
507 greater insight in for instance alveolar simplification [129], Moreover, this will allow us to
508 discern consequences of BPD from early COPD development and treat patients accordingly.

509 Awareness for the need for respiratory follow-up after suffering from early life lung
510 disease is only just emerging and local efforts are putting specific clinics into place. As the
511 prognosis and long-term consequences of early life lung disease are highly variable and

512 cannot be predicted, these initiatives are essential in order to obtain better insight herein. To
513 achieve this, the transfer of clinical data is an important issue. Patients and their data
514 undergo transitions between multiple healthcare providers: from the obstetricians, the
515 neonatologists and paediatricians to adult pulmonologists; and likely also between hospitals
516 using separate databases and systems. Ethics and privacy regulations hinder the connection
517 of these databases. For prospective monitoring, it will be essential to determine which
518 clinical data and possibly biological samples should be collected and stored (*infrastructure*).
519 Prior to establishing standardized prospective follow-up, adult pulmonologists should at least
520 become aware of early life events and exposures of their patients that might have
521 significantly contributed to their current health problems (*education*). This insight into early-
522 life events can offer extremely valuable information to help our understanding of the
523 pathogenesis of adult lung diseases and the heterogeneous representation of each
524 individual condition.

525

526 *Research*

527 A major challenge in a multidisciplinary research approach to early origins of lung
528 disease is connecting basic and translational researchers to understand the clinical
529 conditions and manifestations. Based on the clinical definition of the respective lung disease,
530 various animal models are utilized for these studies. However, not a single model fully
531 represents the complexity of the human situation. Each individual model simulates specific
532 parts of the condition. These models are chosen based on their appropriateness to answer a
533 specific research question and contribute to moving the field forward. However, we need to
534 increase awareness of their limitations (such as phenotypic variability observed in patients)
535 and search for alternatives to tackle them. Furthermore, better representative models for
536 neonates should be established and coupled with *ex vivo* and *in vitro* models, including gut-
537 on-a-chip or lung-on-a-chip.

538 Interdisciplinary approaches and collaborations will increase creativity and often the
539 likelihood for major break-throughs. Here, interdisciplinary does not only relate to

540 technologies, but also refers to the multi-systemic dimensions of lung diseases, the use of
541 multiple exposure models to reflect the complexity of the clinical situation and also
542 encompasses modelling and examination of the extra-pulmonary effects and inter-organ
543 crosstalk and their consequences for the disease. Furthermore, Integration and connection
544 of existing data sets from cohorts (*infrastructure*) should help to decipher gender-, ethnic-,
545 age- and environment-specific effects as well as iatrogenic (for example antibiotics) causes
546 linked to the onset of lung diseases.

547 To investigate how early life exposures and alterations in the microbiome could
548 interfere with developmental, cellular, and immunological processes and ultimately
549 contribute to development of lung disease, it is imperative that the composition of a
550 physiologically 'healthy' lung at different times in early life is defined on these different levels.
551 This would allow for the recognition of altered processes that may predispose to lung
552 disease development. Epidemiological and intervention-studies (*translational/clinical*) are
553 essential to define such physiological "healthy" conditions that lead to for example a health-
554 promoting lung microbiota and absence of lung disease. These studies should highlight the
555 functional role of these processes and the importance of investigating the underlying
556 molecular mechanisms.

557 Finally, translational research should always aim for understanding, prevention and
558 treatment of lung diseases. Endogenous and exogenous risk factors that negatively affect
559 the development of the lung, pulmonary immune system and the lung microbiome and
560 predispose for lung disease should be defined. Subsequently, new targets should be
561 discovered to develop new treatment strategies, including maternal or postnatal nutritional
562 interventions and antimicrobial treatments beyond antibiotics. Definition of nutritional aspects
563 that promotes a healthy gut microbiota could enhance respiratory host defence mechanisms
564 as part of immune homeostasis and decrease viral/bacterial infections/colonisations that
565 may affect lung development, lung immune maturation and lung microbiome composition.
566 Addressing these aims will not only provide new insights in the host-microbe interaction in

567 the context of lung health and disease, but also offer new avenues for early intervention and
568 prevention of respiratory disorders.

569

570 *Infrastructure and tools*

571 Without the proper infrastructure and tools, it is difficult to enhance and join our
572 research efforts. Calls for interdisciplinary funding opportunities are scarce and it is
573 imperative to join forces through collaborative networks. An important opportunity to move
574 the field forward relies on the creation of cohort of patients with biospecimen repository
575 similar to what has been done in the Global Alliance to Prevent Prematurity and Stillbirth
576 (GAPPS) repository (<https://www.gapps.org/>), where the focus has been placed on the
577 sharing of samples. This will allow not only the access to the samples but will also forge a
578 collaborative and potentially pluridisciplinary effort toward achieving common goals to
579 understand the mechanisms underlying the early origins of lung diseases. Moreover,
580 enhanced infrastructure is needed to facilitate the acquisition of cross-country ethical
581 approvals to aid in these efforts.

582 To ensure the acquisition of more reliable, valid and reproducible data in this field
583 between different research groups and countries, it is imperative to develop a consensus
584 analysis framework with standard operating procedures for sample preparation and
585 acquisition, methods, instruments and bioinformatics pipelines. This requires an
586 interdisciplinary network including clinicians, microbiologists, immunologists, molecular and
587 cell biologists, computational scientists, and bioinformaticians.

588

589 **Acknowledgements**

590 On November 11th-12th 2019 scientists from multiple disciplines gathered at a
591 European Research Society sponsored Research Seminar in Lisbon, to discuss current
592 investigations into the role of pre- and postnatal exposures, immune maturation and the
593 influence of microbial composition on lung development and predisposition to disease from a
594 multidisciplinary perspective. This meeting and the discussions that followed resulted in this

595 review, including concerns and recommendations for the future of this multidisciplinary field
596 of research. The authors acknowledge the participants of the ERS Research Seminar ‘Early
597 Origins of Chronic Lung Disease: an interdisciplinary approach’ (Lisbon, November 2019)
598 and the associated scientific symposium at the annual ERS 2019 congress in Madrid, for
599 their participation in the stimulating discussions leading to this review. The authors would like
600 to thank Matthew Randall for his contribution to the illustration of figure 2.

601

602 **Funding sources:**

603 This review is based on a Research Seminar funded by the European Respiratory
604 Society. MAAA is supported by Deutsche Forschungsgemeinschaft (DFG; AL1632/2-1),
605 Marga and Walter Boll Stiftung, Oskar Helene Heim Stiftung. S.K. is supported by the
606 Austrian Science Fund (FWF) Special Research Program Chromatin Landscapes (L-Mac: F
607 6104-B21). REM was supported by the Max Planck Society; the German Center for Lung
608 Research: and German Research Foundation through grants 390649896, 268555672,
609 284237345, 160966624 and 420759458. NLR is supported by the Lung Foundation
610 Netherlands (6.1.16.088). JJPC was supported by a Dirkje Postma Talent Award from the
611 Lung Foundation Netherlands (11.1.16.152).

612

613

614 **Figure legends**

615 **Figure 1. Early life exposures, immune maturation and priming of the developing lung**
616 **for disease.**

617 The prenatal and perinatal environments can have profound effects on the development and
618 progression of respiratory diseases. Different maternal exposures (diet, smoking, medication
619 usage) and maternal inflammation can promote foetal immune programming. Moreover,
620 early life colonization of the lungs is imperative for shaping of the immune response. Early-
621 life changes in lung development, specific environmental exposures and alterations in lung
622 immune maturation following such changes and exposures can lead to the development of

623 childhood and adult respiratory diseases. Alterations in what is considered “healthy” lung
624 development, as can be caused by for example chorioamnionitis associated
625 bronchopulmonary dysplasia (BPD) or aberrant lung structure associated with preterm birth,
626 may prime the neonate, via changes in immune maturation or cellular mechanisms in the
627 lung, for increased susceptibility to develop respiratory complications in later life.

628

629 **Figure 2. Focus areas for future interdisciplinary research into early origins of chronic**
630 **lung disease.**

631 To advance our understanding of the early origins of chronic lung disease four main focus
632 areas and future directions for advancement within these areas have been identified: 1)
633 policy, 2) clinical assessment, 3) research, and 4) infrastructure and tools. Central to these
634 focus areas is education of researchers and clinicians, but also education of and
635 communication to the general public Clinical observations and research findings are required
636 to inform policy making which consequently serves as a basis to inform, guide and educate
637 the public. In order to facilitate proper clinical assessment and both basic and translational
638 research, adequate infrastructure is needed in the form of databases and biobanks.
639 Moreover, interdisciplinary training of professionals (clinicians, midwives, basic and
640 translational researchers, and bioinformaticians and biostatisticians) working across the
641 focus areas is required.

642

643

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