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1 **Childhood asthma heterogeneity at the era of precision medicine:**
2 **Modulating the immune response or the microbiota for the management of asthma**
3 **attack.**

4
5 **Lejeune Stéphanie^{1,2}, Deschildre Antoine^{1,2}, Le Rouzic Olivier^{2,3}, Engelmann Ilka⁴,**
6 **Dessein Rodrigue^{2,5}, Pichavant Muriel², Gosset Philippe²**

7 ¹ CHU Lille, Univ. Lille, Pediatric Pulmonology and Allergy Department. Hôpital Jeanne de
8 Flandre, F-59000 Lille, France.

9 ² Univ. Lille, INSERM Unit 1019, CNRS UMR 9017 CHU Lille, Institut Pasteur de Lille,
10 Center for infection and immunity of Lille, F-59019 Lille cedex, France.

11 ³ CHU Lille, Univ. Lille, Department of Respiratory Diseases, F- 59000 Lille cedex, France.

12 ⁴ Univ. Lille, Virology laboratory, EA3610, Institute of Microbiology, CHU Lille, F-59037 Lille
13 cedex, France.

14 ⁵ Univ. Lille, Bacteriology departement, Institute of Microbiology, CHU Lille, F-59037 Lille
15 cedex, France.

16

17 **Corresponding author:**

18 Philippe Gosset, PhD

19 OpInFIELD, CIIL, Institut Pasteur de Lille

20 1, rue du Pr A. Calmette

21 59019- Lille Cedex –FRANCE

22 Tel : +33 320 87 79 65

23 E-mail: philippe.gosset@pasteur-lille.fr

24

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31 **ORCID Numbers:**

32 S. Lejeune: <https://orcid.org/0000-0001-7009-2113>

33 A Deschildre: <https://orcid.org/0000-0003-2006-7221>

34 O. Le Rouzic: <https://orcid.org/0000-0003-4439-2950>

35 M. Pichavant: <https://orcid-org/0000-0002-1315-4397>

36 P. Gosset: <https://orcid.org/0000-0002-4043-6429>

37

38 **Abstract (249 words)**

39 Exacerbations are a main characteristic of asthma. In childhood, the risk is increasing
40 with severity. Exacerbations are a strong phenotypic marker, particularly of severe and
41 therapy-resistant asthma. These early-life events may influence the evolution and be
42 involved in lung function decline.

43 In children, asthma attacks are facilitated by exposure to allergens and pollutants, but
44 are mainly triggered by microbial agents. Multiple studies have assessed immune responses
45 to viruses, and to a lesser extent bacteria, during asthma exacerbation. Research has
46 identified impairment of innate immune responses in children, related to altered pathogen
47 recognition, interferon release, or anti-viral response. Influence of this host-microbiota dialog
48 on the adaptive immune response may be crucial, leading to the development of biased T
49 helper (Th)2 inflammation. These dynamic interactions may impact the presentations of
50 asthma attacks, and have long-term consequences.

51 The aim of this review is to synthesize studies exploring immune mechanisms impairment
52 against viruses and bacteria promoting asthma attacks in children. The potential influence of
53 the nature of infectious agents and/or preexisting microbiota on the development of
54 exacerbation is also addressed. We then discuss our understanding of how these diverse
55 host-microbiota interactions in children may account for the heterogeneity of endotypes and
56 clinical presentations. Finally, improving the knowledge of the pathophysiological processes
57 induced by infections has led to offer new opportunities for the development of preventive or
58 curative therapeutics for acute asthma. A better definition of asthma endotypes associated
59 with precision medicine might lead to substantial progress in the management of severe
60 childhood asthma.

61 **Introduction**

62 Childhood asthma is a major public health issue in industrialized countries.
63 Development and progression of the disease is marked by exacerbations, also called
64 asthma attacks, which are one of the most prevalent causes of hospitalization in children
65 with consequently significant costs worldwide [1–3]. They constitute key events in the natural
66 history of childhood asthma and are major markers of its heterogeneity [4]. Mechanisms
67 leading to asthma attacks are not fully understood and may differ between children,
68 according to their age range or phenotype.

69 Pediatric literature on asthma usually distinguishes preschool recurrent wheeze / also
70 called preschool asthma in children aged 5 years and younger; from school-age children
71 asthma, in those aged 6 to 11 years; and adolescent asthma, in those aged 12-17 years
72 [3,5,6]. During preschool years, these events can be isolated, with few or no interval
73 symptoms, and their persistence despite conventional maintenance treatment, such as
74 inhaled corticosteroids (ICS), is a marker of severity [6,7]. Although viral infections are the
75 main triggers of acute asthma, early and multiple allergic sensitization is a strong risk factor
76 of presenting severe and frequent exacerbations [7,8]. In school-age children and
77 adolescents, acute asthma attacks are usually less frequent, and not only triggered by lung
78 infections, but also by exposure to allergens and pollutants [3,9,10]. Exacerbations rates
79 increase with the severity of the disease, which is defined according to treatment pressure to
80 obtain asthma control [4,5]. Repetition of frequent and severe episodes under high doses of
81 ICS associated with another maintenance treatment (mainly long acting beta2 agonists) is a
82 criteria of severe asthma in these age-groups [11,12], as demonstrated in pediatric asthma
83 cohorts [13,14]. Additionally, it is now recognized that patients prone to exacerbations
84 constitute a distinct susceptibility phenotype, possibly associated with different endotypes,
85 including specific airway inflammation patterns, or inflammatory mechanisms in response to
86 infectious agents [15]. In some individuals, the severity of the disease and propensity to
87 develop acute asthma may also vary throughout the course of childhood [16]. These early-
88 life events can influence the long-term evolution at adult-age, in terms of asthma

89 persistence, phenotype and alterations of lung function, that may lead to early chronic
90 obstructive pulmonary disease (COPD) [17]. Early interventions could modify the natural
91 history of the disease. However, in children with severe asthma, conventional maintenance
92 therapies may be insufficient to prevent asthma attacks [7,18], and repeated or prolonged
93 oral corticosteroids are associated with many side effects, such as defective growth [19].
94 New treatment strategies are now needed. Interestingly, some studies have shown the
95 efficacy of biologics in pediatric asthma, mainly on severe exacerbations, and suggested
96 disease-modifying properties of some of them, making it a particularly attractive strategy in
97 children [20].

98 Viruses are closely linked to wheezing illnesses during early childhood [21].
99 Respiratory syncytial virus (RSV), and rhinoviruses (RV), both RNA viruses, are among the
100 most common causes of lower respiratory tract infections, *i.e.* bronchiolitis and/or
101 pneumonia, with a predominance of RSV in the first 12 months of life and then RV in the
102 following years [22]. Birth cohort studies and other epidemiological studies have shown a
103 strong association between early RV-induced wheeze, and to a lesser extent, RSV-induced
104 wheeze, and subsequent development of childhood asthma [23–26]. Moreover, pediatric
105 asthma attacks are mainly triggered by respiratory viruses, particularly RV, both in the
106 preschool and school-age [10,27–31]. The question of whether asthma-associated
107 underlying inflammation promotes the pathogenic effect of viral infection or whether the virus
108 induces exaggerated inflammation is still under debate and has been the subject of many
109 research studies over the past 25 years.

110 Alongside viruses, there is also a growing interest on the impact of bacteria in the
111 onset of asthma attacks and the perpetuation of inflammation. Neonatal airway colonization
112 with bacteria including *Haemophilus influenzae*, *Moraxella catarrhalis* and/or *Streptococcus*
113 *pneumoniae*, has been associated with persistence of asthma [32]. Indeed, Kloepfer, *et al.*
114 have shown frequent viral and bacterial co-infection in asthmatic children aged 2-18 years
115 [33]. It is well known that viral infection can predispose to bacterial secondary infection. A
116 few studies have begun to describe inflammatory phenotypes linked with altered microbiota,

117 known as dysbiosis, within the airways [34,35]. Interactions between viruses and bacteria,
118 personal risk factors (e.g. genetic background and atopy), and environmental exposures
119 may promote more severe acute asthma episodes that will influence the progression of
120 asthma [21]. However, the precise role of each of these factors and their interplay with the
121 host immune defenses remains to be elucidated.

122 The aim of this review was to synthesize studies exploring human innate immune
123 mechanisms responses against viruses and bacteria during asthma attacks and to provide
124 hypotheses to decipher how they may contribute to the phenotypes observed in childhood
125 asthma. We will then discuss how therapeutic strategies targeting these pathways may
126 improve the management of acute asthma in children.

127

128 **1/ Impact of viruses on the onset of asthma attacks: chicken or egg?**

129 Up to now, mechanisms explaining susceptibility to develop acute asthma upon
130 respiratory viruses, in particular RV and to a lesser extend RSV, in asthmatic children prone
131 to exacerbation are not fully understood. As innate immune cells play a central role in the
132 onset of anti-viral defenses, they have been the focus of many studies in pediatric and adult
133 asthma, conducted both *ex-vivo* and *in-vivo*, contributing to the identification of new asthma
134 endotypes. The original studies presented in this review have been summarized in **Table 1**.

135

136 **1.1 Virus recognition during asthma attack is mainly driven by airway**
137 **epithelial cells**

138 Before addressing the viral infections impact on asthma attacks, we will briefly
139 describe the virus-induced anti-viral responses. Airway epithelial cells (AEC) are the main
140 cellular targets of pathogens, and act as sentinel cells in response to infection (**Fig 1**) [36–
141 38]. These structural cells are a central part of innate immune response [39]. They first act
142 as a complex physicochemical barrier, *via* the presence of tight intercellular junctions and
143 the muco-ciliary escalator, clearing foreign particles out. If not cleared, Pathogen Associated
144 Molecular Patterns (PAMP) on the surface of infectious agents, will then be recognized
145 through Pattern Recognition Receptors (PRR), in particular Toll-Like Receptors (TLR) and
146 intracellular RNA helicases, which will trigger innate immune responses. Although AEC play
147 an early and central role in orchestrating innate immune defenses, innate immune cells
148 including alveolar macrophages (AM) and dendritic cells (DC), especially plasmacytoid DC
149 (pDC), are also involved in innate immune responses against pathogens [40–44].

150 Studies aiming to explore PRR recognition by epithelial cells and innate immune cells
151 from asthmatic children are multiple but heterogeneous. In cultures of primary AEC from
152 children with severe therapy resistant asthma (STRA), lower expression levels of
153 extracellular and intracellular PRR than healthy controls were observed at baseline [45].
154 Expression of PRR was also decreased upon infection with RV on these cultures and in
155 those from adult asthmatics, suggesting a persistent defect in adulthood [45,46]. Hence, in

156 AM from adults with severe asthma, Rupani, *et al.* also showed a reduction of TLR7
157 expression after RV infection compared with healthy subjects, with an effect potentially
158 mediated by microRNAs [47]. Conversely, in *ex vivo* cultures of cells from broncho-alveolar
159 lavages (BAL) cells and peripheral blood mononuclear cells (PBMC) from adults with mild-to-
160 moderate asthma, no impairment of PRR expression (TLR, RNA-helicases) was observed
161 [48]. This discrepancy was also illustrated by our *in vivo* real-life study conducted in 72
162 school-age children with allergic asthma hospitalized for severe exacerbation, associated
163 with viral infection in 64% (RV: 51%) [49]. Whereas viral infection did not modulate PRR
164 expression and function on PBMC as compared with non-infected asthmatic patients, a
165 reduced expression of PRR was reported in a sub-group of children prone to reinfection
166 outside an exacerbation (steady state 8 weeks later), and displaying neutrophilic airway
167 inflammation away from exacerbation [10,49]. Although present both at exacerbation and
168 steady state, this impairment of PRR expression as well as the inflammatory profile was not
169 associated with the clinical evolution and risk of exacerbations in the year following
170 exacerbation [50].

171 Altogether, these data suggest that impairment of PRR recognition in asthmatic
172 children could be limited to a subpopulation of patients, potentially severe, and may persist
173 into adulthood. Rather than an altered baseline expression or synthesis of PRR, a down-
174 regulation of the expression and activation of the co-receptors, and/or enzymes involved in
175 signaling may be determinant. Longitudinal *in vivo* studies could provide more insights into
176 these mechanisms and allow studying their dynamics during the course of the disease.

177

178 **1.2 Modulation of anti-viral responses**

179 Activation of PRR on AEC and innate immune cells will onset various signaling
180 pathways, such as interferon regulatory factors (IRF) 3 and 7, and nuclear factor-kappa B
181 pathway (NF-kB). This will lead to the production of anti-viral molecules such as interferons
182 (IFN) and anti-microbial peptides, including interferon-stimulated genes (ISG) as well as
183 cathelicidins and defensins [51]. Anti-microbial peptides have immunomodulatory activities

184 and can alter host cellular responses. LL-37, also called human cationic antimicrobial
185 peptide is the only human peptide of the cathelicidin family and one of the most-studied
186 [52,53]. In *in vitro* models, LL-37 has the ability to reduce virus replication, notably for RSV
187 [53,54]. In a population of 77 asthmatic children, Arikoglu, *et al.* have shown that children
188 prone to develop acute asthma attacks displayed higher blood levels of cathelicidins and
189 lower levels of vitamin D at baseline than children with controlled asthma, suggesting a role
190 for anti-microbial peptides in acute asthma [55]. However, the evolution of these baseline
191 levels during acute asthma attacks and their impact on the anti-viral and inflammatory
192 response remain unknown.

193 The majority of studies conducted in asthmatic subjects have had the aim to explore
194 anti-viral IFN response upon viral infection. They have observed a defect of production of
195 type I IFN. In response to RV, most *ex-vivo* studies have demonstrated an impairment of
196 IFN- α and IFN- β production in AEC from pediatric asthmatic patients compared to levels
197 observed in cells from healthy controls [45,56–58]. This impairment was also demonstrated
198 in adult cells [59–61]. Interestingly, similar results have been observed in *ex-vivo* cultures of
199 PBMC. As early as 2002, Bufe, *et al.* have shown in an *ex-vivo* model a defective production
200 of IFN- α in PBMC from pediatric asthmatic patients compared to healthy controls, upon
201 Newcastle disease virus (NDV) infection, an avian respiratory virus belonging to the
202 *Paramyxoviridae* family and well-known potent inducer of IFN- α [62]. They later extended
203 these observations in whole blood cells cultures from adult asthmatics, upon infection with
204 either NDV or RSV [63]. In the later years, similar results have been shown in pediatric
205 studies [64,65] but also in adult studies [45,48,66]. So far, only a few studies have been
206 conducted upon *in vivo* inoculation and natural infection. In a cohort of preschool children,
207 IFN- α systemic levels were reduced at the time of RV-induced exacerbation in asthmatics,
208 as compared with healthy controls during an asymptomatic RV infection [30]. In our recently
209 published cohort of preschool asthmatic children with natural virus-induced exacerbation
210 (RV: 73%), the most severe children, who were prone-to-exacerbation, displayed lower
211 plasma concentrations of IFN- β at exacerbation than the others, suggesting a defect of

212 production in response to viruses limited to this sub-population [8]. In another study
213 conducted in older children, we did not observe altered IFN responses in sputum, plasma
214 and cultures of stimulated-PBMC of patients presenting a virus-induced exacerbation,
215 compared with those with a non-virus-induced exacerbation [49]. In adult asthmatics, a
216 significant type I IFN defect was demonstrated in bronchial epithelium and sub-epithelium
217 obtained from bronchial biopsies compared with healthy controls, after experimental infection
218 with RV-A16 [67]. In contrast, Schwantes, *et al.* described elevated rather than low RNA
219 levels of type I IFN in sputum from adult asthmatic patients, compared to those observed in
220 healthy subjects, early in the course of a virus-induced exacerbation.[68].

221

222 Following their discovery in 2003, there has been increasing knowledge regarding the
223 implication of type III IFN implication in anti-viral responses [69]. *Ex-vivo* studies conducted
224 in AEC from asthmatic subjects have suggested a parallel impairment of IFN- λ 1 and IFN-
225 λ 2/3 production upon RV infection in pediatric patients [45,56,57], and this impairment
226 seemed to persist in adult patients [46,59]. *In vivo*, this defective response was not observed
227 at the time of asthma exacerbation in sputum and/or PBMC from atopic asthmatic children
228 [30,49,70], nor in adults [68,71], suggesting that there are compensatory mechanisms or
229 other sources of IFN, supplementing the defect observed in resident cells.

230 A few studies have explored the potential pathways leading to a defect of IFN
231 production in asthmatic patients. Downregulation of IRF3 or IRF7, following virus infection in
232 asthmatic cells, has been suggested [57,59]. Gielen, *et al.* have also demonstrated a role of
233 the molecule suppressor of cytokine signaling 1 (SOCS1), exercising a negative-feedback
234 on IFN production following viral infection in children with STRA [57].

235 Collectively, these studies suggest a potential impairment of anti-viral IFN response,
236 in particular type I IFN, during asthma attack. Rather than explained by immaturity in the first
237 years of life, this defective response could also be limited to a sub-phenotype of patients
238 who are prone-to-exacerbation, from early childhood to adulthood. The effect of age, the

239 kinetics of the IFN response following viral infection and the impact of viral load in the
240 airways remain to be addressed.

241

242 **1.3 Cytokines and epithelial-derived alarmins (IL-25, IL-33 and TSLP) response against** 243 **viral infection**

244 Airway epithelial cells display immunoregulatory properties by producing pro-
245 inflammatory cytokines, including alarmins interleukin (IL)-25, IL-33 and thymic stromal
246 lymphopoietin (TSLP), released upon viral infection, and implicated in the recruitment of
247 macrophages, DC, T cells and granulocytes, such as eosinophils and neutrophils. This will
248 promote the adaptive immune response through DC maturation and migration to draining
249 lymph nodes associated with the airways. Dendritic cells will thus induce naïve T cell
250 differentiation into antigen-specific T cells. This will drive the adaptive immune response
251 within the airways to clear the virus out. The alarmins also contribute to a biased T helper
252 (Th)2 immune response by activating type 2 innate lymphoid cells (ILC2) and polarizing DC
253 in order to promote T cells differentiation (**Fig 1**). This specific cytokine environment in
254 asthmatic patients favor naïve T cells polarization into Th2 cells, producing IL-4, IL-5, IL-13,
255 as opposed to Th1 cells, producing IFN- γ and TNF- β [37,72]. These cytokines are
256 responsible for the type 2 inflammation and induce the pathophysiological features of
257 asthma, including eosinophil mobilization, mucus hypersecretion, smooth muscle
258 hyperplasia and airflow obstruction [3,73]. . Under the influence of IL-5, eosinophils will
259 enter the airways and perpetuate type 2 inflammation [49,74]. They will also contribute to
260 resolution of immune responses, including tissue repair [75]. Other cytokines, such as type
261 17 cytokines: IL-17, IL-21, IL-22, can also promote mucus hypersecretion and production of
262 cytokines and chemokines by AEC. They will favor the recruitment of neutrophils, through
263 the induction of C-X-C chemokines, conducting to a state of chronic inflammation [67]. In the
264 longer term, IL-17 may contribute to the development of airway remodeling during asthma by
265 enhancing the production of profibrotic cytokines, proangiogenic factors, proteases and
266 collagen [76,77].

267 In children, the influence of respiratory viruses and innate immune mechanisms on
268 the adaptive immune response development may be crucial. First, some respiratory viruses
269 may induce the release of alarmins, and thus favor the development of Th2 inflammation. A
270 recently published study showed that children with RV-positive-bronchiolitis displayed
271 increased IL-4/IFN- γ ratio in nasopharyngeal aspirates [78]. Upon RSV infection, Lee, *et al.*
272 observed a higher production of TSLP by AEC from asthmatic children compared to healthy
273 controls [79]. Hence, some respiratory viruses may induce Th2 inflammation in the airways
274 of children and this may be increased in an asthmatic environment. In a cohort of preschool
275 asthmatic children, ILC markers, such as soluble ST2, were induced *in vivo* following RV
276 infection in preschool asthmatic children, and were correlated with nasal levels of IL-33 [80].
277 In our study of school-age children, at exacerbation, airway inflammation in infected patients
278 was characterized by significantly higher IL-5 concentration and eosinophil count than in
279 non-infected patients [49]. This may not be specific to childhood, as Jackson, *et al.* have also
280 demonstrated an induction of IL-4, IL-5 and IL-13 in BAL following RV inoculation in
281 asthmatic subjects, associated with exacerbation severity [81]. Additional *in vitro*
282 experiments showed that IL-33 directly induced Th2 cytokine production by T cells and ILC2.
283 However, this RV-induced Th2 inflammation could be limited to an asthmatic environment in
284 adults, as suggested by an *in vitro* study reproducing the induction of IL-4 and IL-13 by
285 asthmatic PBMC after co-exposition with RV and IL-33, but showing no effect on PBMC from
286 healthy donors [82]. In addition, exposure to respiratory viruses has also been associated
287 with an increase in Th17 cytokines, as demonstrated in co-cultures of RSV-infected AEC
288 with T cells [83].

289 Interferon production is also closely linked to Th2 and inflammatory cytokines. In
290 asthmatic preschool children prone to exacerbation, we observed simultaneously lower
291 production of Th1 cytokines (IFN- γ), Th2 cytokines (IL-5), pro-inflammatory cytokines (TNF-
292 α), regulatory cytokines (IL-10), and type 1 IFN (IFN- β) during virus-induced exacerbation in
293 a subgroup of preschool children with characteristics of severe asthma and a history of

294 frequent exacerbations [8]. This association seems to persist in adults, as suggested by
295 Parsons, *et al.* reporting a parallel decrease of type III IFNs and of IL-6, (C-X-C motif) ligand
296 (CXCL)8 and CXCL10 in adult asthmatic AEC inoculated with RV [46]. In a murine model of
297 allergic asthma, IRF3 controlled the parallel evolution of IFN and Th2 cytokines during acute
298 episodes induced by allergen exposure [84]. Some adult studies have suggested bi-
299 directional influences during viral infection. Upon RV infection, pre-treatment with Th2
300 cytokines in cultures of AEC may impair TLR3 and IRF3 signaling pathway [59], or type 1
301 and 3 IFN expression [57]. Lower production of type I IFN, displayed by some children, could
302 also reduce the apoptosis of neighbored-infected cells and increase viral loads,
303 consequently amplifying airway inflammation, inflammatory cytokine production and thus,
304 asthma attack severity [58,60].

305 Altogether, these studies confirm close interactions between innate and adaptive immune
306 mechanisms against viruses in an asthmatic environment. Modulating airway inflammation,
307 particularly Th2 inflammation, could therefore directly improve anti-viral responses during
308 asthma attacks in some children.

309

310 **1.4 Viral behavior during asthma attack**

311 Although the impairment of anti-viral responses plays a key role in asthma attack,
312 characteristics of the microorganisms are also important. First, pathogenicity of RV strongly
313 differs according to subtypes, probably because of the implication of different cellular targets.
314 Indeed, infection with RV-C strains, which use cadherin-related family member 3 (CDHR3)
315 as a receptor, may be associated with higher clinical severity [10,31,85], whereas B
316 serotypes seem to be responsible for milder symptoms. Interestingly, single nucleotide
317 polymorphism in the *CDHR3* gene, which influences the efficiency of RV-C to infect its target
318 cells, has been associated with childhood asthma susceptibility [86]. Moreover, different viral
319 strains could have different replication rates and thus induce higher viral loads [87].
320 Rhinovirus C strains could also modulate the airways inflammation, and induce higher levels
321 of Th2 and/or Th17 cytokines, as shown in preschool children with RV-C-induced wheeze

322 [88]. Second, systemic viremia has been observed in children infected with respiratory
323 viruses, such as RV-C, and young age was found to be a risk factor associated with viremia
324 [89]. This state could induce an enhanced inflammation in children by interaction of viruses
325 with systemic immune cells. Finally, viral-coinfection has been frequently observed at
326 exacerbation in asthmatic children, particularly in the preschool years [30,31]. The combined
327 effects of several viruses within the airways may modulate both the innate and adaptive
328 responses.

329 These data demonstrate that, in susceptible children, the nature of respiratory
330 viruses, their spreading, as well as their interactions with the host cells may directly impact
331 the inflammation and the severity of asthma exacerbations.

332

333 **1.5 Emerging role of the airways virome**

334 The role of viral carriage and/or asymptomatic infection at steady state, *i.e.* outside
335 an exacerbation, in asthmatic patients has also been hypothesized. Hence, pediatric studies
336 have shown a frequent virus shedding in children, with RV positive samples observed in 19-
337 29% of unselected asthmatic children and in 15-23% of healthy children [10,28,90,91]. In the
338 previously-cited cohort of preschool children prone to exacerbation, we observed even
339 higher rates, with 67% of positive PCR (51% for RV) at steady state [8]. In 78 school-age
340 children, we also observed that 10% of children had positive RV sampling at exacerbation
341 and steady state, and that RV serotypes were systematically different at both time points,
342 pointing toward reinfection rather than persistence [10]. Consistent with these observations,
343 Bergauer, *et al.* also found 54% of RV at steady state in preschool children with asthma, vs
344 45% in healthy controls [30]. Interestingly, RV carriage in both asthmatics and healthy
345 controls was found to be associated with higher levels of type III IFN at baseline than in
346 subjects without. This increase in baseline inflammation seems to persist in adulthood.
347 Hence, in sputum cells from 57 asthmatic adults, da Silva, *et al.* have shown higher baseline
348 levels of IFN and anti-viral molecules compared with healthy subjects [92]. In all, one could
349 hypothesize that RV persistence in asthmatic patients could induce a pro-inflammatory state

350 and/or a desensitization of PRR, which could reduce the response against pathogens within
351 the airways upon new infection.

352 Alongside RV, other respiratory viruses, could also take part in these processes.
353 Metagenomic analyses have made it possible to identify hundreds of viral species within the
354 airways, constituting the respiratory tract virome [93]. In non-asthmatic children, Wang, *et al.*
355 have shown multiple common epidemic respiratory viruses in children with severe acute
356 respiratory infection, whereas, the virome was less diverse and mainly dominated by the
357 *Anelloviridae* family in healthy children [94]. *Anelloviruses* are major components of the
358 virome and display immunomodulatory properties on both innate and adaptive immunity [95].
359 Although their presence has been associated with an impaired lung function in asthmatic
360 children [96], to our knowledge, no study has evaluated their association with asthma
361 attacks. Thus, the contribution of these viruses and their interactions with both pathogenic
362 infectious agents, as well as commensal bacteria from the pulmonary microbiota on chronic
363 inflammation and the onset of acute asthma remain to be determined.

364

365 **2/ The emerging role of pathogenic bacteria and microbiota in asthma**

366 Increasing evidence indicates that the respiratory microbiota, including bacterial and
367 viral microorganisms has an important role in respiratory health and disease [97,98]. In
368 addition, recent data underline the role of bacterial infections in the development and
369 progression of asthma, as summarized in **table 2**.

370 During the first weeks of life, the respiratory tract begins to develop a niche-specific
371 community pattern, where *Staphylococcus aureus* and *Corynebacterium* replace the
372 originally colonizing bacteria and become the dominant microorganisms [98]. The most
373 dramatic changes occur during the first 2 months. In the following six months, the respiratory
374 microbiota continues to mature; relative abundance of *S. aureus* declines, and an increase in
375 *Moraxella*, *Streptococcus*, *Haemophilus*, *Dolosigranulum*, *Alloiococcus*, and *Prevotella sp.* is
376 observed. This period is crucial and alterations of the microbiota have been implicated in the
377 development and the manifestations of chronic lung diseases. .

378

379 **2.1 Bacteria and asthma development**

380 Using bacterial culture techniques, it has been demonstrated that neonates born to
381 asthmatic mothers with upper airway colonization with *S. pneumoniae*, *M. catarrhalis*, and/or
382 *H. influenzae* were at higher risk of presenting asthma at the age of 5 years [32]. This was
383 associated with an aberrant immune response, with increased IL-5 and IL-13 synthesis in
384 PBMC obtained at the age of 6 months [99]. Interestingly, evaluation of the gut microbiota in
385 infants included in the Canadian (CHILD) study showed that infants at risk of asthma
386 exhibited transient alterations of the microbiota, known as dysbiosis, in neonates [100]. More
387 specifically, the relative abundance of the commensal bacterial genera *Lachnospira*,
388 *Veillonella*, *Faecalibacterium*, and *Rothia* was significantly decreased in children at risk of
389 asthma. Furthermore, antibiotic-induced gut dysbiosis has been shown to facilitate the
390 development of allergic asthma in experimental murine models and clinical studies [101]. In
391 a population-based cohort, it has been reported that pre- and post-natal antibiotic exposure
392 was associated with a higher asthma risk [102]. No significant difference in bacterial diversity

393 was observed between samples from those with wheeze and healthy controls. Age and
394 attendance at day care or kindergarten were important factors in driving bacterial diversity
395 whereas wheeze and viral infection were not found to be significantly associated to the
396 bacterial communities. In the Avon Longitudinal Study of Parents and Children (ALSPAC)
397 cohort, children for whom antibiotics course was reported before the age of 2 years were
398 more likely to have asthma at age 7, although microbiota was not characterized [103].
399 Similarly, in British Columbia (Canada), "controlled" antibiotic use during infancy was
400 associated with a reduction in the incidence of pediatric asthma, and was related with the
401 preservation of gut microbial communities [104]. Altogether, these data confirm the need to
402 preserve the microbiota in early infancy in order to limit the development of asthma.

403

404 **2.2 Relationship between bacteria and asthma attack**

405 Bacterial infections potentially contribute to the severity of symptomatic respiratory
406 viral infections and asthma attacks in children. Indeed, there are bidirectional interactions
407 between viruses and airway bacteria that appear to modulate the severity of illness and the
408 likelihood of asthma attack [20]. Pediatric studies have shown that upper respiratory tract
409 colonization with *Streptococcus*, *Haemophilus*, and/or *Staphylococcus* genus during RSV
410 and RV infection increases the risk of hospitalization independently of age and of the
411 presence of asthma [105,106]. In our previously-cited cohort of preschool children, asthma
412 attacks were frequently associated with viral infection (94%, mostly RV). Pathogenic bacteria
413 were isolated in induced sputum in 56%, mainly *H. influenza*, *M. catharralis* or *S.*
414 *pneumoniae* [21]. In adult asthmatics, asymptomatic RV infection was associated with
415 increased abundance of *Dolosigranulum* and *Corynebacterium*, whereas symptomatic RV
416 infection seemed to be related with a higher frequency of *Moraxella* [107]. In another adult
417 study, co-infection with virus and bacteria induced higher levels of cytokine/chemokine than
418 bacterial infection [108]. Interestingly, circulating CCL2 and CCL5 concentrations were
419 increased in infected asthmatic patients compared with non-asthmatic patients.
420 *Streptococcus* species may also influence the course of asthma exacerbation, as shown by

421 Kama, *et al.* in preschool children [109]. Indeed, patients with *S. pneumoniae* colonization in
422 the pharyngeal microbiota had significantly shorter wheezing episodes and reduced lung
423 inflammation (including lower levels of TNF- α).

424 In summary, these data suggest an ability for bacteria to promote asthma attacks and
425 modulate the inflammation during childhood. Targeting bacterial infections during asthma
426 attacks could thus be an alternative strategy in some children.

427

428 **2.3 Dysbiosis and impact on asthma natural history**

429 Early-life dysbiosis of the microbiota, in particular lung microbiota, may have long-
430 term consequences on asthma natural history. During viral infection in children less than two
431 years old, detection of *Moraxella* was found to be associated with current wheeze at 5 years
432 old and presence of *Streptococcus*, *Moraxella*, or *Haemophilus* was associated with a high
433 risk of asthma development [110]. Mansbach, *et al.* showed in a joint modeling analysis
434 adjusting for 16 covariates, including viral trigger, that a higher relative abundance of
435 *Moraxella* or *Streptococcus* species 3 weeks after day 1 of hospitalization for severe
436 bronchiolitis was associated with an increased risk of recurrent wheezing [111]. In contrast,
437 no significant difference in microbiota composition was observed between oro-pharyngeal
438 microbiota from children with acute wheezing (0-14 years old) and healthy controls [112]. In
439 this study, the large range of patient ages and day care attendance were important
440 confounding factors, since the microbiota evolves throughout childhood.

441 In school-age asthmatic children, Zhou, *et al.* assessed by longitudinal
442 measurements the relationship between nasal airway microbiota and either loss of asthma
443 control, or risk of severe exacerbations [113]. Whereas the commensal
444 *Corynebacterium/Dolosigranulum* cluster characterized the patients with the lowest risk of
445 disease progression, a switch towards the *Moraxella* cluster was associated with the highest
446 risk of severe asthma exacerbations. Similarly, in another cohort of school-age asthmatic
447 children, nasal microbiota dominated by *Moraxella* species was associated with increased
448 exacerbation risk, and eosinophil activation at exacerbation [35]. *Staphylococcus* or

449 *Corynebacterium* species–dominated microbiota were linked with reduced respiratory illness
450 and exacerbation event rates, whereas *Streptococcus* species-dominated microbiota
451 increased the risk of RV infection. Interestingly, using unbiased airway microbiome-driven
452 clustering, Abdel-Aziz, *et al.* recently described in adult asthmatics two distinct robust severe
453 asthma phenotypes that were associated with airway neutrophilia, and not related to the
454 presence of *Moraxella* species [114]. Robinson, *et al.* assessed the lower airway microbiota
455 by performing BAL at steady state in severe preschool wheezers [34]. They reported two
456 profiles: a *Moraxella* species “dysbiotic” cluster, associated with airway neutrophilia and a
457 “mixed microbiota” cluster associated with macrophage- and lymphocyte-predominant
458 airway inflammation, thus suggesting that bacteria might influence the characteristics of lung
459 inflammation.

460 Altogether, these data show that airway dysbiosis in early life might contribute to
461 recurrent wheeze and asthma, and to the severity of the disease. Among bacterial species,
462 the early presence of *M. Catharralis* and *H. influenzae* during childhood seems to be a
463 marker of evolution toward a severe disease, through the modulation of the inflammatory
464 reaction. In contrast, colonization with *S. pneumoniae* might have some anti-inflammatory
465 effects. However, the role of bacteria is often exacerbated or modulated by intrinsic (atopy,
466 inflammation characteristics) or extrinsic factors, such as viral infections and antibiotics.

467

468

469 **3/ Consequences on asthma phenotypes and endotypes**

470 It is now clear that phenotyping and even more so, endotyping asthmatic patients is
471 an essential step to understand the diversity of immune responses at the time of an asthma
472 attack. We will review the commonly described endotypes of childhood asthma, successively
473 based on airway leukocyte infiltrate, profiles of allergic sensitization and nature of asthma
474 trajectories.

475 Among airway inflammatory phenotypes, neutrophilic asthma appears to be a distinct
476 endotype, associated with asthma severity in adults, and possibly involved in corticosteroid
477 insensitivity [76]. In children, several studies have shown that association of airway
478 neutrophilia with severity is inconstant [115–118], and may not persist throughout the course
479 of the disease [116,119]. However, neutrophils present in the airways of severe asthmatic
480 children may have enhanced survival and proinflammatory functions that could increase
481 baseline inflammation [120]. As previously stated, airway neutrophilia has also been
482 associated with a different IFN response, at baseline and at the time of asthma attack, both
483 in adults [66,92] and pediatric studies [49]. This could have an impact on lung infections.
484 Thus, we have shown that a subgroup of school-aged children with positive viral PCR both
485 at baseline and exacerbation displayed airway neutrophilia [49]. In severe adult asthmatics,
486 airway neutrophilia has also been associated with lesser microbiota diversity in BAL
487 [114,121]. Finally, experimental RV infection in adult asthmatic subjects directly induces
488 bronchial mucosal neutrophilia [122]. Therefore, the neutrophilic endotype may be
489 characterized by an alteration of airway microbiota and association with viral infections.

490 Airway eosinophilia is highly frequent in asthmatic children, observed in severe and
491 non-severe asthma, often but not always associated with response to ICS, thus highlighting
492 the diversity of eosinophilic profiles in pediatric asthma [116,118,123]. Mouse models and
493 adult studies have suggested a role of IL-5 induced airway eosinophilia as a positive
494 regulator of RV receptor intercellular adhesion molecule (ICAM)-1 on AEC, and a negative
495 regulator of PRR and antiviral responses [124,125]. However, in children, it remains unclear
496 whether airway eosinophils are involved in the pathophysiology of severe asthma, and in the

497 propensity to develop acute asthma attacks [126]. In addition, peripheral blood eosinophilia,
498 currently used as the basis for selection of eosinophil-targeted biologics, may not be
499 representative of airway eosinophilia in children [127]. Most importantly, it has been reported
500 that eosinophilia is not necessarily associated with higher levels of Th2 cytokines [118], and
501 other cells such as alarmin-stimulated ILC2 may have a greater biological importance in the
502 pathophysiology of asthma attacks [128]. Eosinophils can also have beneficial effects in
503 childhood asthma, as they display regulatory functions and contribute to lung repair and
504 mucosal homeostasis [75]. Peripheral blood eosinophilia has recently been identified has a
505 biomarker predicting the decrease of severity throughout the adolescent years [16]. Finally, it
506 remains to be determined whether eosinophil-targeted biologics, such as anti-IL-5
507 mepolizumab, are efficient in reducing asthma attacks in children [129]. Overall, as opposed
508 to adults, airway eosinophilia does not appear as the preferential biomarker to classify
509 pediatric patients nor to assess their risk of acute asthma attacks.

510 Rather than through the recruitment of eosinophils, type 2 inflammation may play a
511 key role in some children's susceptibility to develop acute asthma attacks through IgE
512 sensitization. Hence, atopy, defined as allergic sensitization, is a key feature associated with
513 risk of asthma attacks in childhood [7,8,14]. As shown by latent-class analyses, atopy is also
514 one of the main factors that may account for the heterogeneity of the disease, especially in
515 the preschool years [130,131] and could drive response to treatment [3]. Pre-existing atopy
516 may play a role in the modulation of IFN response upon viral infection in children, although
517 studies have shown conflicting results [59,61,65]. Durrani, *et al.* have suggested a direct
518 implication of the high-affinity IgE receptor (FcεRI) in this defect, as its cross-linking on pDC
519 from asthmatic allergic patients is associated with reduced IFN secretion upon RV infection
520 [65]. Following virus-induced exacerbation in atopic children, upregulation of FcεRI receptor
521 on monocytes and DC was observed, associated with a concomitant upregulation of pro-
522 inflammatory cytokines, including IL-4 and IL-13 [132], suggesting that respiratory viral
523 infection in atopic children may initiate an atopy-dependent cascade that amplifies virus-
524 induced airway inflammation. This could initiate long-term remodeling and inflammation.

525 Hence, IgE sensitization, especially if occurring early in life and multiple, has been identified
526 as the main factor associated with long-term asthma risk (recurrence of exacerbations,
527 persistence of asthma, impaired lung function) in neonatal cohorts, including MAAS (Great
528 Britain), MAS (Germany), PARIS (France) and PASTURE (Europe) [133–136].

529 Describing asthma trajectories in neonatal longitudinal cohorts may precisely allow
530 identifying novel endotypes. For instance, in a birth cohort of children at high risk of atopy,
531 cord blood cells were tested for their ability to produce IFN after exposure to polyinosinic-
532 polycytidylic acid (Poly I:C), a double stranded RNA that mimics viral PAMP [137]. A group
533 of low producers of type I and III IFN response upon stimulation representing 24% of the
534 patients was identified. They displayed higher risk of lower respiratory tract infection and
535 persistent wheeze at the age of 5, suggesting that these clinical outcomes are partly
536 determined by constitutional mechanisms.

537 Altogether, beyond airway leukocyte and type 2 inflammation, childhood asthma
538 endotypes seem to be associated with the characteristics of airway microbiota and of the
539 associated immune response. Because these might involve dynamic processes, the study of
540 asthma trajectories throughout childhood is crucial. A better characterization of asthma
541 endotypes will be an essential step in order to achieve a personalized medicine.

542

543 **4/ In the future: machine learning and systemic biological approaches**

544 Digital tools provide an innovative way to study the integrated immune response in
545 asthmatic patients. For instance, Custovic, *et al.* used machine learning in an *in vitro* model
546 of RV-A16 infection on PBMC extracted from 11 years old children and described 6 clusters
547 of cytokine response to infection [138]. One cluster, with patients from whom PBMC
548 displayed low IFN responses, high pro-inflammatory cytokines, low Th2 responses and
549 moderate regulatory responses, was associated with early-onset asthma and the highest risk
550 of asthma exacerbations and hospitalizations for lower respiratory tract infection.

551 Omic technologies, known as genomic, epigenomic, transcriptomic, proteomic,
552 single-cell omics, metabolomics, microbiomics, exposomics, are others fast-developing

553 tools, generating a massive amount of data reflecting the biological state of cell populations
554 at a given time, that will certainly make possible in the future to identify new endotypes and
555 their biomarkers [139,140]. For instance, with the development of the Human Genome
556 Project, genome-wide association studies have allowed to link childhood asthma with
557 association signals in or near genes involved in innate immune response pathways, such as
558 the *TLR1* locus, and the *IL-33* locus [141,142]. Transcriptomics analyses, which aim at
559 investigating all RNA transcripts in a biological sample, will certainly enable the identification
560 of a large quantity of genes modulated in certain conditions. A recent study has described 94
561 distinct gene-expression modules between virus-induced and non-virus-induced
562 exacerbation in nasal and sputum cells from a large cohort of school-age children [143]. In
563 asthma attacks, this tool may also provide information on the activated pathways linked with
564 inflammatory and anti-microbial response [144,145]. RNA sequencing in nasal lavage cells
565 from asthmatic children at exacerbation led to identify some genes overexpressed as
566 relevant “nodes” linking genes of the inflammatory response, such as IRF7 [144].
567 Transcriptomic analyses have also been used to identify genes associated with different
568 phenotypes of asthmatic patients [146]. Finally, metabolomics, the study of the metabolites
569 produced in living systems (both humans and microbiota) represents another promising
570 strategy in the upcoming years to address the role of immuno-metabolism as well as lung
571 and gut microbiota in asthma pathophysiology [147,148].

572 To sum up, systemic approaches integrating all biologic pathways are now
573 increasingly used to study the mechanisms leading to susceptibility to acute asthma attacks
574 in children. They may facilitate the identification of novel multimodal biomarkers linked with
575 specific endotypes and therapeutic targets.

576

577

578

579 **5/ Therapeutic strategies modulating innate immune defenses during asthma attacks**

580 Conventional maintenance treatment, such as ICS, may be insufficient to prevent
581 attacks in children, especially in severe asthma. Severe therapy-resistant asthma is
582 precisely characterized by repeated severe exacerbations despite high dose daily treatment.
583 As the knowledge about infection-underlying pathophysiological processes grows, new
584 therapeutic approaches that may contribute to either improve innate immune responses or
585 limit the occurrence and consequences of infections during asthma attacks have been
586 recently explored (**Figure 2**).

587

588 **5.1 Immunostimulatory therapeutics**

589 As we have shown earlier, asthmatic children may display an impairment of PRR
590 signaling that leads to susceptibility to infection-induced asthma attacks. One of the
591 strategies to enhance their anti-viral responses could involve stimulation of PRR. This
592 strategy has only been tested in pre-clinical and adult studies. Following promising results
593 from pre-clinical studies [149], Silkoff, *et al.* reported results of a clinical trial assessing the
594 effect of CNTO3157, a TLR3 agonist, upon *in vivo* inoculation of RV-A16 in adult subjects
595 with mild-to-moderate asthma [150]. The molecule was ineffective in blocking the effect of
596 RV-16 challenge on the onset of asthma exacerbation, without effect on asthma symptoms
597 nor lung function. Additionally, all post-inoculation exacerbations occurred in the CNTO3157
598 group, and two were severe, thus pointing toward a lack of efficacy and safety of the TLR3
599 targeting drugs.

600 Another way of enhancing PRR activation and activate different pathways could
601 therefore be the administration of PAMP-like adjuvants or particles. Some teams have
602 hypothesized that Poly I:C, a double stranded RNA that activates TLR3 and intracellular
603 receptors, might modulate anti-viral responses. Preclinical studies have shown contrasting
604 results, with promising effect in the context of Influenza A virus infection [52,151], but
605 concerns regarding its safety. Indeed, Tian, *et al.* have described, in a mouse model, a
606 prolonged IFN response upon *S. pneumonia* and *S. aureus* infection, inducing an impaired

607 bacterial clearance [152]. We have also demonstrated in a murine model of allergic asthma
608 that TLR3 ligands can enhance the Th2 response [153]. Thus, the exact consequences of
609 administering exogenous TLR ligands (including TLR7-8) in humans are still unknown and
610 their potential role in bacterial secondary infections in the lungs need to be addressed.

611 Another immunomodulatory strategy, particularly appealing in pediatric asthma, could
612 rely on the administration of bacterial lysates [154]. Two main therapies have been
613 described: polyvalent mechanical bacterial lysates (PMBL®) and Broncho-Vaxom OM-85
614 BV. They contain lysates of 8 pathogenic bacteria including *H. influenzae* and *S.*
615 *pneumoniae*. Both PMBL® and OM-85 administration have proven to be efficient in
616 preventing respiratory tract infections in adult and pediatric patients with chronic bronchitis
617 and/or history of recurrent chronic infections [155–159]. Interestingly, in a trial conducted in
618 elderly COPD patients, Ricci, *et al.* observed a reduced number of seroconversion against
619 numerous viral pathogens in patients treated with PMBL®, as well as a better control of the
620 number of all infectious episodes and COPD exacerbations, suggesting that the preventing
621 effect of PMBL® on acute infections was not only on bacterial infections, but also on viral
622 infections [160]. In 152 school-aged children with allergic asthma, Emerick, *et al.* have
623 described a reduction of exacerbations after 12 weeks of treatment with PMBL®, and no
624 serious adverse event, although no improvement of asthma control scores were achieved
625 [161]. In 75 preschool children with recurrent wheeze, Razi, *et al.* demonstrated that OM-85
626 BV reduced the rate and duration of wheezing episodes in the 12 months following the
627 treatment initiation, and reduced the number of acute respiratory tract illnesses [162]. Finally,
628 in toddlers aged 6-18 months, a clinical trial is ongoing to assess the preventive effect of
629 OM-85V on the onset of first wheeze episode related to lower respiratory tract infection
630 during a 3 years observational period [163]. Regarding the mechanisms of action, prevention
631 of acute respiratory infections was not induced by purified LPS or bacterial DNA, suggesting
632 that other PAMP contained in bacterial lysates would activate innate immunity and lead to
633 promotion of type 1 inflammation by DC [164–166].

634 Altogether, immunostimulatory therapeutics may represent a promising strategy as
635 additional treatments to prevent asthma attacks but their safety and efficacy need to be
636 properly addressed. Because multiple microorganisms may be involved in acute asthma, it
637 remains also unclear whether they would have an impact on the onset and severity of acute
638 asthma attacks in children.

639

640 **5.2 Therapeutics enhancing anti-viral responses**

641 Anti-microbial peptides, among which LL-37, are key molecules of the innate immune
642 responses against both viruses and bacteria. Thus, it has been hypothesized that
643 supplementing with LL-37 or inducing its production among AEC could enhance anti-
644 infectious defenses. Sousa, *et al.* have tested the effect of exogenous cathelicidin on RV-1B
645 replication after inoculation on cultures of AEC [167]. They reported that exogenous LL-37,
646 as well as homologous porcine cathelicidins, either administered prior or after infection,
647 displayed antiviral activity against RV by reducing the metabolic activity of infected cells.
648 However, one preclinical study in a mouse model of asthma showed *in vivo* a deleterious
649 effect of exogenous cathelicidin-related antimicrobial peptide in airway inflammation and
650 responsiveness [168], and to our knowledge, there is no published study *in vivo* in humans.
651 Nevertheless, promising results regarding vitamin D supplementation on the release of LL-
652 37 by AEC in *in vitro* experiments have been published [169–171]. In clinical studies, there is
653 evidence toward a protective effect of vitamin D supplementation on risk of acute respiratory
654 infections in children [172]. Although there are other effects of vitamin D that might explain
655 its clinical efficacy [80,173], there is a well-studied biological link between vitamin D and host
656 microbial peptides, *via* the translocation of vitamin D to the nucleus of AEC, where it
657 activates Vitamin v responsive elements in promoter regions of anti-microbial peptide genes
658 such as LL-37 [52,171,174]. Hence, targeting the vitamin D / cathelicidin axis could
659 represent a promising strategy during acute asthma in children.

660 As studies have shown deficient IFN responses in asthmatic patients, it has been
661 hypothesized that IFN supplementation upon viral infection could enhance anti-viral

662 responses and reduce asthma attacks severity. Interferon therapies (pegylated IFN- α in
663 association with ribavirin) have first been developed in other diseases such as in hepatitis C
664 and human immunodeficiency virus [175,176]. Ribavirin is a synthetic guanosine nucleoside
665 that impairs the synthesis of viral mRNA [52]. Its clinical utility, upon respiratory virus
666 infection, has been shown in RSV, Adenovirus, RV, or in (SARS)-CoV immunocompromised
667 patients [177–180]. Since exogenous IFNs reduced RV replication in *in vitro* cultures of RV-
668 infected AEC from asthmatic patients [181], a trial was initiated in 147 adult asthmatic
669 patients developing symptoms of viral infection (68% RV) to evaluate the efficacy of inhaled
670 IFN- β treatment for 14 days [182]. Overall, it did not improve asthma symptoms, but it
671 enhanced morning peak expiratory flow recovery and reduced the need for additional
672 treatment, *i.e.* oral corticosteroids or antibiotics. In an exploratory analysis of the subset of
673 more difficult-to-treat patients, IFN supplementation improved asthma control questionnaires.
674 In addition, it boosted anti-viral innate immunity with persistent elevated serum levels of
675 CXCL10, and decreased levels of pro-inflammatory molecules such as CCL4 and CXCL8. In
676 the INEXAS phase 2 trial, designed to assess efficacy of inhaled IFN- β 1a for 14 days to
677 prevent asthma exacerbations in adults, McCrae, *et al.* also reported improvement in the
678 morning peak expiratory flow in the treated arm compared with placebo, but the study
679 stopped early [183,184]. No study has yet been conducted in pediatric patients.

680 Collectively, anti-viral therapies could represent potential therapies in the treatment of
681 acute asthma attacks in children. However, the exact timing of administration upon viral
682 infection and onset of acute asthma, and the population of children who might benefit from
683 these strategies remain to be determined.

684

685 **5.3 Role of anti-microbial therapeutics**

686 Apart from ribavirin, other anti-viral therapies, such as capsid-targeting molecules
687 pleconaril, BTA-798 and V-073 have been studied in enteroviruses infections. However,
688 none of these compounds have proven sufficient efficacy and safety in clinical trials [185]. In
689 particular, a phase 2 clinical trial with intranasal pleconaril in children aged 6 years and

690 older, and adults, showed no impact of treatment on the onset of RV-induced cold symptoms
691 or acute asthma exacerbation [186]. Although their efficacy needs to be addressed in clinical
692 trials, DNzyme therapies could also be promising strategies, as recently suggested by an *in*
693 *vitro* study showing good catalytic activity of 2 candidates (Dua-01 and Dua-02) on various
694 RV strains [187].

695 As described above, bacteria and microbiota may be associated with asthma
696 development and attacks, and antibacterial therapies have emerged as alternative strategies
697 to prevent and/or to treat asthma exacerbation [154,188,189]. Treatments aiming to
698 influence the composition of the microbiota are presently tested in different clinical situations
699 and recent data have suggested that these methods might have some interest in asthma.
700 Transplantation of healthy microbiota seems a promising alternative as demonstrated in gut
701 inflammatory diseases [190]. In preclinical study, Arrieta, *et al.* demonstrated that oral
702 inoculation with four bacterial species previously identified as associated with risk of
703 childhood asthma to germ-free mice improved allergic airway inflammation in their adult
704 offspring [100]. Several studies have also evaluated the benefit of prebiotic and probiotic but
705 their interest during childhood asthma attack has yet to be evaluated [191,192]. Another
706 option may be to administer bacteria metabolites, which can mimic the effect of normal
707 microbiota. Thus, feeding pregnant mice with short chain fatty acid (SCFA), such as acetate,
708 was associated with modulation of the maternal microbiota and protection from developing
709 allergic airway disease in the offspring [193]. Finally, an increasing number of studies in
710 experimental models of lung infections has demonstrated the potential interest of fecal
711 microbiota transplantation and/or SCFA supplementation, suggesting that they might be
712 efficient in bacteria- and virus-induced exacerbations [194–196].

713 The potential modulatory effects of some antibiotics on asthma attacks in children are
714 subject of intense research. First, in terms of prevention, Schwerk, *et al.* observed a positive
715 impact of long-term antibiotics (mainly amoxicillin and amoxicillin-clavulanic acid) in
716 preschoolers with severe wheeze who had positive bacterial infection on BAL samples, with
717 reduced rates of exacerbations and hospitalizations at 6 months follow-up compared with

718 rates before treatment [197]. Second, immunomodulatory effects of macrolides are well-
719 described [198], and have been evaluated in the treatment of acute asthma in children.
720 Hence, in the Early Administration of Azithromycin and Prevention of Severe Lower
721 Respiratory Tract Illnesses in Preschool Children (APRIL) study, Bacharier, *et al.* conducted
722 a placebo-controlled trial in 607 preschool children with recurrent wheeze and no controller
723 medication, testing the efficacy of azithromycin started prior to the development of lower
724 respiratory tract illness [199]. Macrolide administration reduced the risk of progressing to
725 severe lower respiratory tract illness (*i.e* requiring a course of oral steroids), without
726 evidence for induction of azithromycin-resistant organisms, nor adverse events. In the
727 Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC2010) cohort,
728 children with recurrent asthma-like symptoms, some of whom under maintenance treatment,
729 were randomly assigned to receive either azithromycin or placebo in case of episode of
730 asthma-like symptoms, lasting at least 3 days [200]. Children treated with azithromycin
731 displayed a significant reduction of episode length, and the effect size increased with early
732 administration of treatment. Moreover, Mandhane, *et al.* conducted a placebo-control trial in
733 preschool children aged 12 to 60 months, presenting to the emergency department with
734 wheeze, to assess the effect of azithromycin [201]. They did not show a reduction of duration
735 of respiratory symptoms in the treated group, neither in the time to the next exacerbation
736 upon 6 months follow-up. Thus, there is still limited evidence on the use of azithromycin as a
737 preventive treatment of acute asthma attacks [202]. In addition, studies have been
738 conducted in adult patients to test the effect of azithromycin as a preventive strategy, with
739 conflicting results [203–205]. Notably, Gibson, *et al.* described in a placebo-controlled trial
740 that 48 weeks maintenance treatment with azithromycin decreased the frequency of asthma
741 exacerbations and improved asthma-related quality of life in symptomatic asthma despite
742 current maintenance treatment [204]. In a meta-analysis of the three main studies conducted
743 in adult patients [203–205], maintenance treatment with azithromycin was associated with a
744 reduced rate of exacerbations in asthma as well as in all subgroups (non-eosinophilic,
745 eosinophilic and severe asthma), and a good tolerance [206]. Examining each exacerbation

746 type separately, patients with eosinophilic asthma reported fewer oral corticosteroid courses,
747 and patients with non-eosinophilic and severe asthma reported fewer antibiotic courses.
748 Additionally, a specific effect of macrolides in neutrophilic asthma had been reported
749 previously by Simpson, *et al.* in adult subjects with severe refractory asthma, treated with
750 clarithromycin for 8 weeks [207]. Clarithromycin therapy reduced sputum concentrations of
751 IL-8 and neutrophil numbers leading to lower levels of neutrophil elastase and Matrix
752 metalloproteinase (MMP)-9 concentrations versus placebo. Finally, a decrease of
753 inflammatory cytokines, notably IL-1 β , IL-6 and IL-33, was observed *in vitro* in cultures of
754 human AEC from asthmatic patients pretreated with clarithromycin, in a context of RV14
755 infection, suggesting a direct anti-viral effect [208]. Consistent with this hypothesis, another
756 *in vitro* study showed an effect of azithromycin to reduce RV replication after inoculation in
757 AEC, associated with an increase in IFN and interferon-stimulated genes mRNA expression
758 and protein production [209].

759 To sum up, although macrolides and more particularly azithromycin constitute an
760 appealing strategy in acute asthma, further studies are needed to identify the phenotypes of
761 potential responders and the possible adverse events. Since antibiotics have the potential
762 limitation of eliminating commensal bacteria that protect from asthma development, it must
763 be emphasized that their use should only be considered in particular clinical situations [188].

764

765

766 **5.4- Potential role of biologics targeting type 2 inflammation in anti-microbial** 767 **response**

768 We have highlighted the close interactions between underlying airway inflammation,
769 host anti-microbial responses and microorganisms leading to asthma attacks. Therefore,
770 novel therapeutics targeting inflammatory pathways in asthma, such as anti-Th2
771 inflammation biologics, may have a specific impact on lung infections.

772 Omalizumab is an anti-IgE monoclonal antibody, targeting the effector mechanisms
773 of Th2 inflammation. After proof of efficacy in pediatric clinical trials, it has been widely

774 prescribed in severe asthmatic atopic children aged older than 6 years, providing more
775 insights on the longer-term evolution [210]. In real-life studies, we have observed higher
776 levels of asthma control after its initiation, and a significant decrease of asthma attacks in
777 children previously prone to exacerbation [211]. Hence, a direct effect of omalizumab on RV-
778 induced exacerbations in children has been suggested [212,213]. In the Preventative
779 Omalizumab or Step-up Therapy for Severe Fall Exacerbations (PROSE) study, a placebo-
780 controlled trial conducted among 478 inner-city asthmatic children aged 6 to 17 years with 1
781 or more recent exacerbations, Esquivel, *et al.* observed a decrease of the duration of RV
782 infections, viral shedding, and risk of RV-induced illnesses in omalizumab-treated patients
783 [212]. A recently published adult study including results from two clinical trials has compared
784 the effect of omalizumab on the response to an experimental inoculation with RV-16 in
785 allergic asthmatic adults [214]. Omalizumab-treated patients displayed a reduction of
786 symptoms of lower respiratory tract infection, more pronounced in the four first days
787 following inoculation, suggesting that the protective effect of omalizumab on virus-induced
788 exacerbations occurs in the early stages of infection. Teach, *et al.* described other results
789 from the PROSE study that compared omalizumab with placebo and omalizumab with an
790 inhaled corticosteroid boost with regard to fall exacerbation rates when initiated 4 to 6 weeks
791 before return to school [215]. They recorded 86 exacerbations, among which 89% were
792 associated with a respiratory tract virus detection, most commonly RV (81%). More
793 importantly, they observed a significantly lower fall exacerbation rate in the omalizumab
794 group, but no difference between omalizumab and inhaled corticosteroid boost except in the
795 subgroup of patients with exacerbation during the run-in phase. In an *ex vivo* study of PBMC
796 taken during the intervention phase, the authors observed increased IFN- α responses
797 following RV-A16 inoculation in the omalizumab-treated group, suggesting that the
798 mechanism of Omalizumab protection against exacerbations could involve IFN- α dependent
799 antiviral effect [20]. Another study in children with exacerbation-prone asthma before and
800 during omalizumab treatment described the effect of *ex vivo* inoculation of RV and Influenza
801 A virus on PBMC and blood pDC, after IgE cross-linking on cell-surface Fc ϵ RI [216]. The

802 authors observed that omalizumab increased both virus-induced IFN- α responses in PBMCs
803 and pDC, and subsequently reduced Fc ϵ RI expression on pDC. Altogether, the lower
804 asthma exacerbation rates consecutive to omalizumab treatment were related to IFN- α
805 responses and attenuated Fc ϵ RI expression on pDC.

806 Omalizumab remains the most-studied biologic in pediatric asthma but others have
807 been experimented in the past years [217]. Mepolizumab, an anti-IL-5 antibody therapy, has
808 proven to be efficient in reducing the exacerbation rate in adults and is now available in
809 children older than 6 years old with eosinophilic asthma in Europe and the US [218]. As
810 previously stated, its efficacy in reducing exacerbation rates in pediatric clinical trials and
811 real-life studies remain to be determined [129]. However, adult studies suggest it may have a
812 modifying effect on RV-induced inflammatory response. Hence, a placebo-controlled trial
813 was conducted in adult patients with mild asthma, randomized to receive mepolizumab
814 versus placebo, followed by RV-16 inoculation, to study its impact on anti-viral responses
815 [219]. The authors showed that mepolizumab attenuated baseline blood eosinophils
816 numbers and activation status, but did not prevent the activation of remaining eosinophils
817 upon RV infection. However, after infection, it enhanced levels of airway B lymphocytes and
818 AM and modified BAL levels of CCL20 and IL-1RA. Effects of other drugs targeting Th2
819 cytokines and alarmins, including lebrikizumab (anti IL-13), dupilumab (anti IL-4R),
820 reslizumab (anti-IL-5), benralizumab (anti-IL-5R), tezepelumab (anti-TSLP), or anti-IL-33
821 monoclonal antibodies are currently studied in pre-clinical and clinical studies [217].

822 In the next years, new studies are needed regarding these novel treatments, both in
823 allergic and non-allergic asthmatic children, to provide new evidence on their immunological
824 effects and potential disease-modifying properties, and to fully determine the
825 phenotypes/endotypes of good responders. Most importantly, specific studies in the
826 youngest children need to be carried out, with appropriate end-points [129]. This effort will
827 contribute to enter the era of precision medicine in pediatric asthma.

828

829

830 **6. Conclusion**

831 In conclusion, asthma attacks are a main feature of pediatric asthma, especially of
832 severe asthma. We have highlighted studies suggesting impairment of the mucosal anti-
833 microbial immune responses during acute asthma and/or at steady state. Interestingly, these
834 alterations seem to inconstantly persist into adulthood. These various altered pathways may
835 reflect different endotypes underlying asthma heterogeneity and consequently, constitute
836 targets for new treatment approaches. Preventing, understanding and efficiently managing
837 acute asthma attacks remain one of the unmet needs in severe childhood asthma, especially
838 in the youngest children. At the era of precision medicine, early interventions targeting
839 mucosal immune responses, lung infections, and microbiota, in early childhood, could have
840 disease-modifying properties and positive impact on the trajectory of childhood asthma.

841

842

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849

850

851 **CrediT:**

852 Lejeune S: Conceptualization, Data collection Data analysis, Writing, Review & Editing,
853 Deschildre A: Conceptualization, Data collection Data analysis, Writing, Review & Editing,
854 Le Rouzic O: Data collection Data analysis, Writing, Review & Editing,
855 Engelmann I: Data collection Data analysis, Writing, Review & Editing,
856 Dessein R: Data collection Data analysis, Writing, Review & Editing,
857 Pichavant M: Data collection Data analysis, Writing, Review & Editing,
858 Gosset P: Conceptualization, Data collection Data analysis, Writing, Review & Editing.

859

860 **Figure Legends:**

861 **Figure 1: Summary of the main mechanisms favoring asthma development and**
862 **involved in asthma attack.**

863 These mechanisms (**in red**) involve: (1) Impairment of innate immune responses; (2)
864 Influence of the host-microbiota dialog on Th2 inflammation; (3) Pathogen characteristics; (4)
865 Airway leukocyte inflammation. These dynamic interactions may impact the presentations of
866 asthma attacks, and have long-term consequences.

867

868 AM: Alveolar macrophages; AEC: airway epithelial cells; DC: dendritic cells; IFN: Interferon;
869 IL: Interleukin; ILC2: type 2 innate lymphoid cells; IRF: interferon regulatory factor; PAMP:
870 Pathogen-associated molecular pattern; PRR: pattern recognition receptor; RV: Rhinovirus;
871 TSLP: Thymic stromal lymphopietin.

872

873 **Figure 2: Possible therapeutics strategies to limit asthma development/progression**
874 **and attacks.**

875 These strategies (**in green**) target: (1) Enhancement of the innate immune responses; (2)
876 Anti-infectious therapeutics and strategies to modulate the microbiota; (3) Alarmins and anti-
877 Th2 biologics.

878 AM: Alveolar macrophages; AEC: airway epithelial cells; DC: dendritic cells; IFN: Interferon;
879 IL: Interleukin; ILC2: type 2 innate lymphoid cells; IRF: interferon regulatory factor; PAMP:
880 Pathogen-associated molecular pattern; PRR: pattern recognition receptor; RV: Rhinovirus;
881 SCFA: Short Chain Fatty Acid, TSLP: Thymic stromal lymphopoietin.

882

883 **Capsule summary:**

884 *Characteristics of host-microbiota interactions in asthmatic children favor asthma attacks*
885 *and may allow to define asthma endotypes. This knowledge offers promising perspectives to*
886 *treat asthmatic children using precision medicine.*

887

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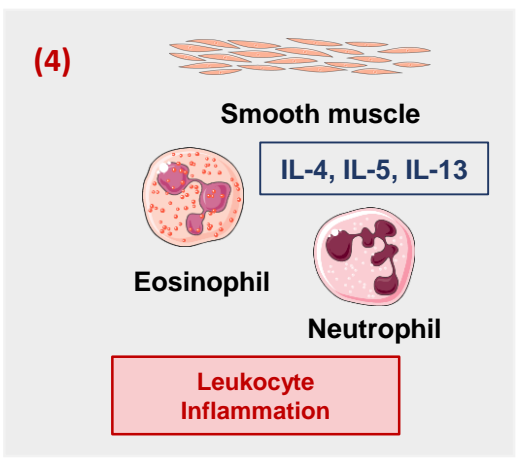
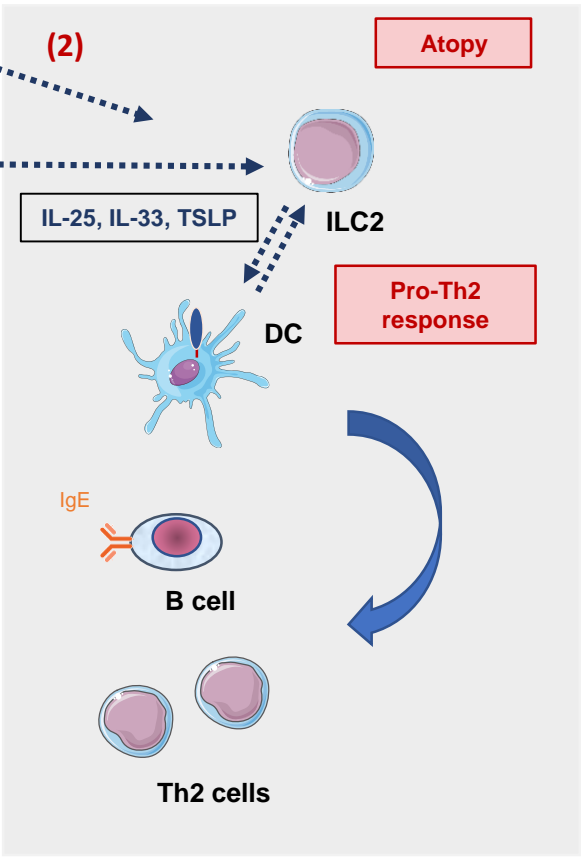
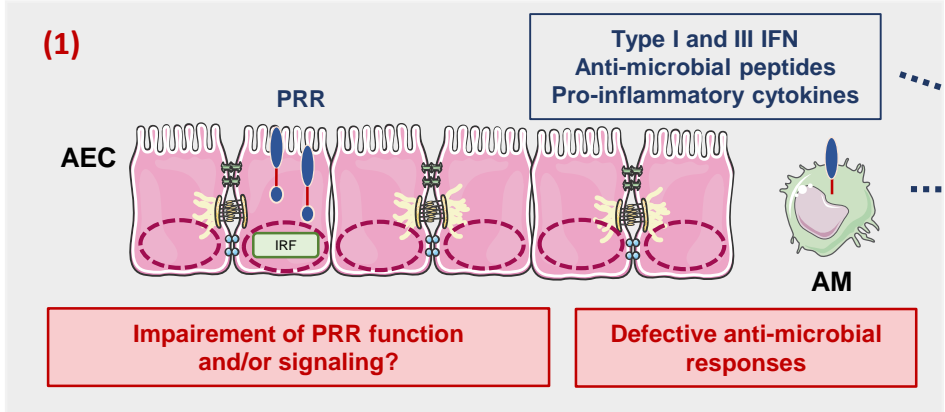
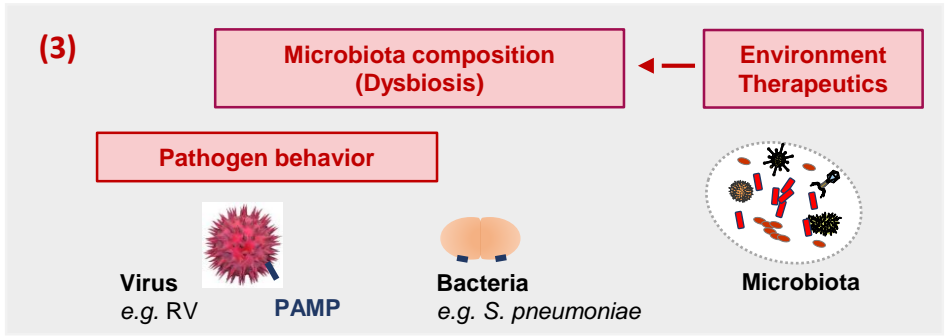
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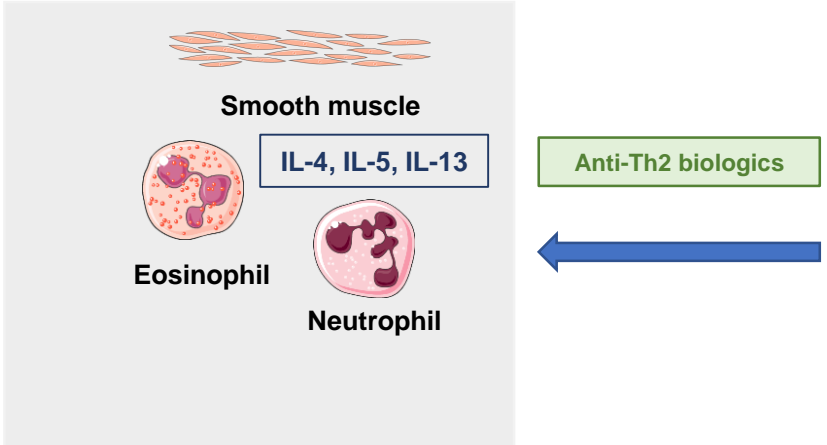
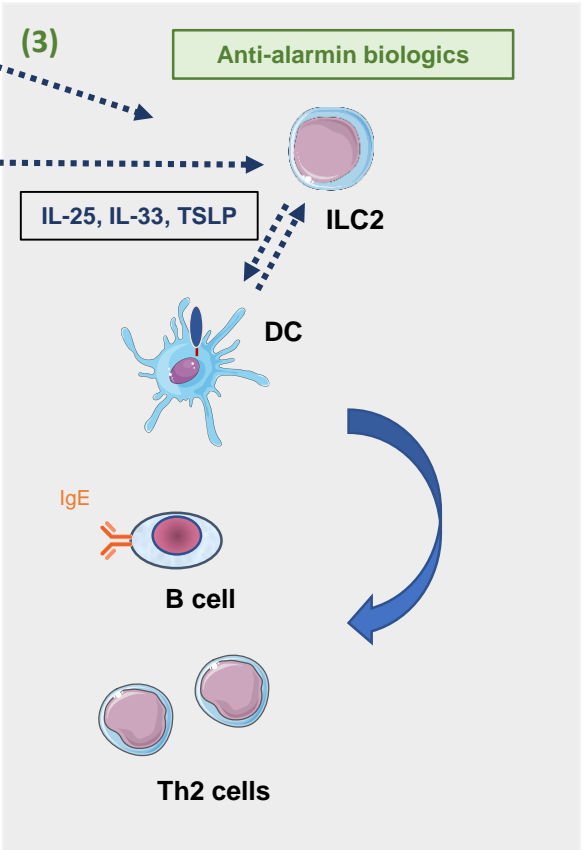
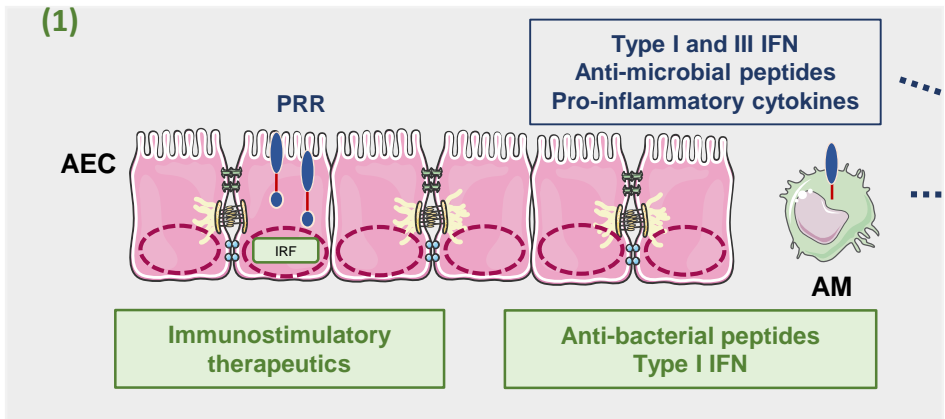
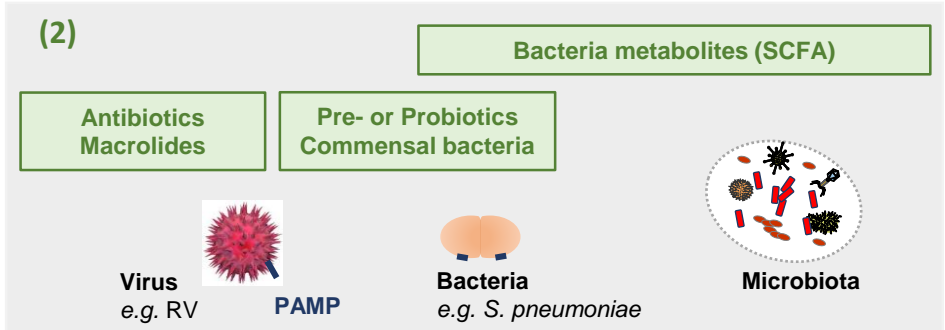
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| Compartment | Study | Design and age-group | Main results | |
|-------------|------------------------------------|---------------------------------------|--|--|
| PRR | <i>In vitro and ex-vivo</i> | | | |
| | AEC | Edwards M, et al. 2013 [45] | RV-A16 Children: <u>Severe therapy resistant asthma (STRA)</u> 11y (9-15y) vs healthy controls | In STRA: lower basal levels of TLR3, reduced RIG-I, MDA5 levels after RV stimulation; lower basal levels of TLR3, reduced RIG-I, MDA5 levels after RV stimulation No association with age, IgE levels, allergen reactivity, BAL/sputum neutrophils and eosinophils, lung function |
| | AEC | Parsons KS, et al. 2014 [46] | RV-B1 Adults: <u>asthma</u> / Healthy controls | In asthma: no impairment of expression of MDA5, TLR3 and reduced release of CXCL-10 |
| | AEC | Contoli M, et al. 2015 [59] | RV-A16 Pre-treatment with IL-4 and IL-13 prior to infection Adults: <u>atopic rhinitis</u> / non-atopic healthy | In atopic cells: impaired immune response to RV-16 in the presence of IL-4 and IL-13, through inhibition of TLR3 expression and signaling (IRF3), |
| | AEC | Moskwa S, et al. 2018 [61] | PIV-3 / RV-B1 Adults: <u>atopic asthma</u> / <u>non-atopic asthma</u> / healthy controls | <u>Atopic asthma:</u> After PIV3 infection: higher IFN- λ 1, IFN- α , IFN- β and IRF7 expressions After RV1B infection: IFN- β higher than in <u>non-atopic asthmatics</u> |
| | AM | Rupani H, et al. 2016 [47] | RV-A16 Pre-treatment with Imiquimod (TLR7 agonist) Adults: <u>severe asthma</u> / Healthy controls | In severe asthma: reduced TLR7 expression, inverse correlation with number of exacerbations Increased levels of 3 microRNAs (miR-150, miR-152, and miR-375) Restored TLR7 expression after blocking these microRNAs with anti-miRNA oligonucleotides |
| | BAL cells PBMC | Sykes A, et al. 2012 [48] | RV-A16 Adults: <u>mild-to-moderate allergic asthma</u> vs healthy controls | In asthma: no impairment of expression of PRR (TLR, RNA-helicases), their adaptor proteins, transcription factors downstream |
| | <i>In vivo</i> | | | |
| | Sputum Serum PBMC | Deschildre A, et al. 2017 [49] | School-age children 8.9 y (6-16y) <u>Atopic asthma:</u> virus vs no virus Severe exacerbation (64% virus, 51% RV) / steady state (8 weeks later) (25% virus, 11% RV) 24% virus at both time | Virus at exacerbation: similar TLR3, RIG-I and MDA-5 expression on blood monocytes and DC, similar levels of IFN- β , IFN- γ , IFN- λ 1 in sputum and plasma, higher airway IL-5 and eosinophils counts than patients with negative viral PCR Virus at both time: modification in PRR expression/function on PBMC, higher airway neutrophilic inflammation at steady state |
| | Bronchial biopsies | Zhu J, et al. 2019 [67] | <i>In vivo</i> inoculation - RV-A16 Young adults: <u>atopic asthma</u> (23 y \pm 1.4) vs non-atopic control subjects (27 y \pm 2.3) | In asthma: PRR expression (TLR3, MDA5, RIG-I) not deficient at baseline; and induced after RV both in epithelium and sub-epithelium |

| <i>In vitro</i> | | | |
|----------------------------|-------------------------------------|--|---|
| AEC | <i>Wark PA, et al. 2005 [60]</i> | RV-A16 Adults: <u>Asthma</u> vs healthy controls | In asthma: Impaired IFN- β , late virus release and late cell lysis, impaired apoptotic response |
| AEC | <i>Edwards M, et al. 2013 [45]</i> | RV-A16 Children: <u>Severe therapy resistant asthma (STRA)</u> 11y (9-15y), vs healthy controls | In STRA: IFN- β , IFN- λ 1, IFN- λ 2/3 mRNA levels lower and RV virus load higher No association with age, IgE levels, allergen reactivity, BAL/sputum neutrophils and eosinophils, lung function |
| AEC | <i>Gielen V, et al. 2015 [57]</i> | RV-B1 / RV-A16 Pre-treatment with IL-4 / IL-13 Children: <u>STRA</u> 11y (9-15y), vs healthy controls | In STRA: SOCS1 expression increased and related to IFN deficiency, increased viral replication Suppression of RV-induced IFN promoter activation in AEC by SOCS1, dependent on nuclear translocation |
| AEC | <i>Parsons KS, et al. 2014 [46]</i> | RV-B1 Adults: <u>asthma</u> / Healthy controls | In asthma: reduced release of IL-6, CXCL-8 and IFN- λ in response to RV, reduced release of CXCL-10 |
| AEC | <i>Kicic A, et al. 2016 [58]</i> | RV-B1 / RV-B14 Children: <u>Asthma</u> 8.2 y (2.6-14.8) vs healthy children 8.4 y (3.2-15.6) | In asthma: greater virus proliferation and release, reduced apoptosis and wound repair (RV-B1), lower IFN- β , higher inflammatory cytokine production Addition of IFN- β : restored apoptosis, suppressed virus replication and improved repair of AEC from asthmatics, no effect on inflammatory cytokine production |
| AM | <i>Rupani H, et al. 2016 [47]</i> | RV-A16 Pre-treatment with Imiquimod (TLR7) Adults: <u>severe asthma</u> / Healthy controls | In severe asthma: reduced IFN responses Increased levels of 3 microRNAs (miR-150, miR-152, and miR-375): Blocking these microRNAs with anti-miRNA oligonucleotides increased IFN production |
| BAL cells | <i>Sykes A, et al. 2012 [48]</i> | RV-A16 Adults: <u>mild-to-moderate allergic asthma</u> vs healthy | In asthma: induction of type I IFN delayed and deficient, associated with airway hyperresponsiveness |
| Bronchial biopsy specimens | <i>Baraldo S, et al. 2012 [56]</i> | RV-A16 Children, 5y \pm 0.5: <u>asthma, atopy</u> / <u>Asthma, no atopy</u> / <u>Atopy, no asthma</u> / No atopy nor asthma (Controls) | In all groups vs controls: reduced type I and III IFN production, increased RV viral RNA IFN inversely correlated with airway eosinophils, IL-4, epithelial damage, total IgE (type III IFN) |
| Whole blood cultures | <i>Bufe A, et al. 2002 [62]</i> | NDV Children: <u>Allergic asthma</u> / non-allergic asthma / allergic rhinitis / healthy control | In allergic asthma: lower virus-induced IFN- α in allergic asthma/ rhinitis: higher production of IFN- γ |
| PBMC | <i>Gehlhar K, et al. 2006 [63]</i> | RSV-1A / NDV Adults: <u>Allergic asthma</u> vs healthy controls | In allergic asthma: significant reduction of virus-induced IFN- α release, independent of virus used; no influence of medication (cortico-steroids) |
| PBMC | <i>likura K, et al. 2011 [64]</i> | RV-B14 Wheeze / healthy; Young children: 2-6y; Youth group: 7-19 y; Adult group: \geq 20y | Asthma (youth group): lower IFN- α production Asthma (youth group and adults): lower IL-6, TNF- α , IL-10 and sFasL productions Wheeze (young children): lower IL-10 associated with persistent wheeze at 2 y follow-up |
| PBMC | <i>Sykes A, et al. 2012 [48]</i> | RV-A16 Adults: <u>mild-to-moderate allergic asthma</u> vs healthy controls | In asthma: no impairment of type I IFN in PBMC, as opposed to BAL cells |
| PBMC | <i>Durrani S, et al. 2012 [65]</i> | RV-A16 Children 10-12 y : <u>allergic asthma</u> / <u>non-allergic asthma</u> / <u>airborne sensitization without asthma</u> / healthy controls. | Allergic asthma: lower IFN-responses after cross-linking Fc ϵ R1 (vs sensitization and vs controls); higher surface expression of Fc ϵ R1 on pDC and mDC Inverse relationship between total IgE and diminished IFN secretion, only when cross-linking of Fc ϵ R1 |
| PBMC | <i>Simpson J, et al. 2016 [66]</i> | RV-B1 Adults: <u>asthma</u> , 76% atopic. Sputum phenotypes: eosinophilic / neutrophilic / paucigranulocytic / granulocytic | In neutrophilic asthma: significantly less IFN- α than PBMC from eosinophilic and paucigranulocytic asthma after RV; IFN- α inversely correlated with serum IL-6, sputum IL-1 β Sputum neutrophils and dose of inhaled corticosteroids independent predictors of reduced IFN- α |

| | | <i>In vivo</i> | | |
|-------------------------|------------------------------------|---------------------------------------|---|--|
| Type I and type III IFN | Bronchial biopsies | Zhu J, et al. 2019 [67] | In vivo inoculation - RV-A16 Young adults: atopic asthma (23 y ± 1.4) vs non-atopic control subjects (27 y ± 2.3) | In asthma: IFN α / β deficiency in epithelium, at baseline, day 4 and week 6 post-inoculation; correlated with viral load, and clinical severity Sub-epithelium: lower frequencies of monocytes / macrophages expressing IFN α / β after RV infection in sub-epithelium; subepithelial neutrophils were the source of IFN α / β |
| | BAL monocytes and AM | Contoli M, et al. 2006 [71] | In vivo inoculation - RV-A16 Adults: <u>severe asthma</u> (<i>ex vivo</i>) / mild asthma without steroids treatment (<i>ex vivo</i> and <i>in vivo</i>) vs healthy controls | In asthma / <i>Ex vivo</i> : Impaired induction and production of type III IFN- λ 1 and IFN- λ 2/3 in AEC and AM, impaired induction of IFN- λ upon LPS stimulation In asthma / <i>in vivo</i> : severity of symptoms, BAL virus load, airway inflammation, reduction of lung function inversely correlated with <i>ex vivo</i> production of IFN- λ |
| | Nasal washes | Miller EK, et al. 2012 [70] | Natural infection: 82% virus Children 5-18 y Upper respiratory infection; <u>Wheezing</u> / no-wheezing RV in 56% wheezing / 37% non-wheezing | Specific association between RV infection and asthma exacerbation, but no difference in virus titers, RV species and inflammatory or allergic molecules between RV+ wheezing and non-wheezing children In RV+ wheezing children: IFN- λ 1 level higher, increased with worsening symptoms |
| | Nasal washes | Kennedy J, et al. 2014 [29] | Natural infection: 74 children (4-18 y): acute wheezing (57% RV) / acute rhinitis (56% RV) / controls Inoculation (RVA-16): 24 young adults Asthma / healthy | Natural infection: No difference in viral loads and levels of IFN- λ 1 between wheezing and acute rhinitis; In children with wheezing: lower levels of sICAM-1 Inoculation: No difference in viral load between groups, levels of sICAM-1 correlated with RV levels |
| | Nasal washes Induced sputum | Schwantes E, et al. 2014 [68] | Natural infection Adults: <u>asthma</u> vs healthy 17 exacerbations : 67% RV | In asthma, at exacerbation: sputum RNA levels of IFN- α 1, IFN- β 1 and IFN- γ correlated with exacerbation and the peak Asthma Index, early in the course of infection, higher levels of IL-13, IL-10 |
| | Serum PBMC | Bergauer A, et al. 2017 [30] | Ex vivo inoculation of RV-1B on PBMC cultures In vivo natural infection Preschool children 4-6y <u>Asthma</u> at baseline (54% RV) / Asthma at exacerbation (100% RV) vs healthy controls | Ex vivo: induction of STAT1/STAT2 by RV in asthma / IRF9 in controls / IRF1 in both Natural infection (baseline): No increase of IFN- α expression in RV+ asthma patients compared with RV+ healthy controls; increase of IFN- λ protein with RV, both in asthma patients and controls Natural infection (exacerbation): reduced serum IFN- α at exacerbation compared with RV healthy children at baseline |
| | Sputum Serum PBMC | Deschildre A, et al. 2017 [49] | School-age children 8.9 y (6-16y) <u>Atopic asthma:</u> virus vs no virus Severe exacerbation (64% virus, 51% RV) / steady state (8 weeks later) (25% virus, 11% RV) / 24% virus at both time | Virus at exacerbation: similar levels of IFN- β , IFN- γ , IFN- λ 1 in sputum and plasma, higher airway IL-5 and eosinophils Virus at both time: lower levels of IFN- γ in plasma and sputum at exacerbation, higher airway neutrophilic inflammation at steady state |
| | Sputum Plasma | Lejeune S, et al. 2020 [8] | Preschool children 30 mo (1y-5y) <u>Recurrent wheeze / preschool asthma;</u> 47% atopy; 70% prone to exacerbation (≥ 2 exacerbations in the previous year) <u>Severe exacerbation</u> (94% virus, 73% RV) / steady state (8 weeks later) (67% virus, 50% RV) | Children prone to exacerbation: lower plasma concentrations of IFN- β and CXCL10 at exacerbation, and lower plasma levels of CXCL10 at steady state |

| | | | | |
|------------------------|-----------------------------------|------------------------------------|---|--|
| Alarmins and cytokines | <i>In vitro</i> | | | |
| | Co-culture AEC and T cells | Qin L, et al. 2011 [83] | RSV-A2 AEC T cells extracted from adult PBMC (healthy volunteers) | Prolonged RSV infection in AEC induced T cells differentiation in Th2 and Th17 subsets that released IL-4, IFN- γ and IL-17 induced by supernatants from RSV-infected AEC |
| | AEC | Lee HC, et al. 2012 [79] | RSV-A AEC from healthy children and asthmatic children | Production of TSLP after infection of AEC with RSV, via activation of an innate signaling pathway that involved retinoic acid induced gene 1, interferon promoter-stimulating factor 1, and nuclear factor- κ B. Greater levels of TSLP after RSV infection in AEC from asthmatic children than from healthy children |
| | PBMC | Jurak L, et al. 2018 [82] | RV-A16 and IL-33 co-exposition Adults with <u>allergic asthma</u> vs healthy controls | In asthmatic cells: IL-5 and IL-13 release induced by RV, enhanced by IL-33, surface protein expression of ST2 induced by IL-33 Predominant source of IL-13 release: ILC2 In healthy cells: IL-33 had no effect on IL-5 and IL-13 production |
| | <i>In vivo</i> | | | |
| | BAL PBMC | Jackson D, et al. 2014 [81] | <i>In vivo</i> inoculation <i>Ex vivo</i> study: AEC, PBMC (T cells, ILC2) Adults: <u>mild-to-moderate asthma</u> vs non-atopic healthy volunteers | <i>In vivo:</i> IL-4, IL-5, IL-13 induced by RV in BAL in asthma Type 2 cytokines and IL-33 correlate with clinical outcomes and viral load <i>Ex vivo:</i> IL-5 and IL-13 production directly induced by IL-33 present in RV-infected AEC supernatants by human ILC2 and T cells |
| | PBMC Serum Nasal washes | Haag P, et al. 2018 [80] | Preschool children 4-6y <i>Ex vivo</i> study: RVB-1 inoculation <i>In vivo:</i> <u>asthma</u> (54% RV) / controls (45% RV) at <u>baseline</u> | <i>Ex vivo:</i> Induction of ILC2 genes in asthma following RV inoculation <i>In vivo:</i> Down regulation of sST2 in asthma and controls by RV Up regulation of sST2 in RV- asthma patients with low levels of 25(OH)-VitD3 In asthma: direct correlation of serum sST2 with nasal IL-33 |
| | Nasopharyngeal aspirates | Yuan XH, et al. 2020 [78] | Children aged less than 14y <u>Bronchiolitis or pneumonia</u> 19.7% RV, 35 bronchiolitis and 31 pneumonia cases | RV bronchiolitis: increased IL-4/IFN- γ and decreased TNF- α /IL-10 ratios, compared with RV pneumonia |
| | Sputum Plasma | Lejeune S, et al. 2020 [8] | Preschool children 30mo (1y-5y) <u>Recurrent wheeze / preschool asthma</u> ; 47% atopy; 70% prone to exacerbation (≥ 2 exacerbations in the previous year) Severe exacerbation (94% virus, 73% RV) / steady state (8 weeks later) (67% virus, 50% RV) | Children prone to exacerbation: lower plasma concentrations of IFN- γ , IL-5, TNF- α , IL-10, and lower levels of IFN- γ in sputum at exacerbation At steady state, lower plasma levels of IFN- γ |

1

2 Table 1: Major studies (pediatric and adult population) assessing immune responses to viral infection during asthma exacerbation

3 *Studies conducted in animal models, and/or without administration of viral particles (e.g. administration of TLR agonists, double-stranded RNAs) were not*
4 *included in this table.*

5 AEC: Airway epithelial cells; AM: Alveolar macrophages; BAL: broncho-alveolar lavage; CCL: C-C motif ligand; CXCL: C-X-C motif ligand; Fc ϵ R1: high-affinity IgE receptor; IgE: Immunoglobulin E;
6 IFN: Interferon; IL: Interleukin; ILC2: type 2 innate lymphoid cells; IRF: interferon regulatory factor; mDC: myeloid dendritic cells; LPS: Lipopolysaccharides ; MDA-5: melanoma differentiation-associated

7 protein 5; NDV: Newcastle disease virus; PBMC: peripheral blood mononuclear cells; pDC: plasmacytoid dendritic cells; PBMC: Peripheral blood mononuclear cells; PCR: Polymerase chain reaction;
8 PIV3: parainfluenza virus type 3; PRR: pattern recognition receptor; RIGI-I: retinoic-acid inducible gene I; RSV: Respiratory syncytial virus; RV: Rhinovirus; sFasL: Serum Soluble Fas Ligand; sICAM-
9 1: Soluble intercellular adhesion molecule-1; siRNA: small interfering RNA; SOCS1: Suppressor Of Cytokine Signaling 1; sST2: soluble suppression of tumorigenicity 2 (IL-33 receptor IL1RL1); STAT:
10 signal transducer and activator of transcription; STRA: severe therapy resistant asthma; Th2: T helper 2; TLR: Toll like receptor; TNF: Tumor necrosis factor.

| | Compartment | Study | Design and age-group | Main results |
|--------------------|----------------|---|--|---|
| Asthma development | Hypopharynx | <i>Bisgaard H, et al. 2007 [32]</i> | Hypopharyngeal samples were cultured from 321 neonates at 1 month of age | 21% of children were colonized with <i>S. pneumoniae</i> , <i>M. catarrhalis</i> , <i>H. influenzae</i> , or a combination of these organisms. Colonization with one or more of these organisms, but not colonization with <i>S. aureus</i> , was significantly associated with persistent wheeze. Neonates colonized in the hypopharyngeal region with one or a combination of these organisms, were at increased risk for recurrent wheeze and asthma early in life. |
| | Gut microbiota | <i>Arrieta MC, et al. 2015 [100]</i> | 319 children enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study | Children at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life. Relative abundance of the bacterial genera <i>Lachnospira</i> , <i>Veillonella</i> , <i>Faecalibacterium</i> , and <i>Rothia</i> was significantly decreased in children at risk of asthma. This reduction in bacterial taxa was accompanied by reduced levels of fecal acetate. Inoculation of germ-free mice with these four bacterial taxa ameliorated airway inflammation in their adult progeny. |
| | Lung | <i>Loewen K, et al. 2015 [102]</i> | 213,661 mother-child dyads with a median follow-up time of 9.3 years from birth. Maternal antibiotic use was determined from records of oral antibiotic prescriptions | 36.8% of children were exposed prenatally to antibiotics and 10.1% developed asthma. Prenatal antibiotic exposure was associated with an increased risk of asthma. Maternal antibiotic use during 9 months before pregnancy and 9 months postpartum were similarly associated with asthma. |
| | | <i>Hoskin-Parr L, et al. 2013 [103]</i> | 4,952 children from the Avon Longitudinal Study of Parents and Children (ALSPAC). Child antibiotic use and asthma, eczema and hay fever symptoms were reported | Children reported to have taken antibiotics during infancy (0-2 yr) were more likely to have asthma. The risk increased with greater numbers of antibiotic courses. The number of courses was also associated with a higher risk of eczema and hay fever but not atopy. |
| | Gut microbiota | <i>Patrick DM, et al. 2020 [104]</i> | 2,644 children from the Canadian Healthy Infant Longitudinal Development (CHILD) prospective birth cohort and among them, 917 were analyzed for fecal microbiota | Reduction in asthma incidence over the study period was associated with decreasing antibiotic use in the first year of life. Asthma diagnosis in childhood was associated with infant antibiotic use (adjusted odds ratio [aOR] 2.15), with a significant dose-response; α -diversity of the gut microbiota was associated with a 32% reduced risk of asthma at age 5 years. |
| Acute asthma | Nasopharynx | <i>Mansbach JM, et al. 2016 [106]</i> | The MARC-35 prospective cohort study of 1,016 infants (age <1 year) hospitalized with bronchiolitis and followed for the development of recurrent wheezing | RSV infection was associated with high abundance of <i>Firmicutes</i> and <i>Streptococcus</i> and a low abundance of <i>Proteobacteria</i> and the genera <i>Haemophilus</i> and <i>Moraxella</i> . RV infection was associated with low <i>Streptococcus</i> and high <i>Haemophilus</i> and <i>Moraxella</i> . RSV/RV coinfections had intermediate abundances. |
| | Nasopharynx | <i>De Steenhuijsen P, et al. 2016 [105]</i> | 106 children less than 2 years of age with RSV infection / 26 asymptomatic healthy control | 5 nasopharyngeal microbiota clusters were identified, characterized by high levels of either <i>Haemophilus influenzae</i> , <i>Streptococcus</i> , <i>Corynebacterium</i> , <i>Moraxella</i> , or <i>Staphylococcus aureus</i> . RSV hospitalization were positively associated with <i>H. influenzae</i> and <i>Streptococcus</i> . RSV-induced expression of IFN-related genes is independent of the microbiota cluster |
| | Nasopharynx | <i>Kloepfer KM, et al. 2017 [107]</i> | 17 adult subjects collected nasal mucus samples on a weekly basis for 5 consecutive weeks | Asymptomatic RV infections were associated with a significant increase in the abundance of <i>Dolosigranulum</i> and <i>Corynebacterium</i> . RV infection preceded the increased odds of detection of <i>Streptococcus</i> and <i>Moraxella</i> by 1 week. |
| | Blood | <i>Giuffrida LM, et al. 2017 [108]</i> | 14 asthmatic and 29 non-asthmatic adult patients with acute respiratory infection 10 healthy individuals | Higher blood concentrations of CCL2 and CCL5 in infected asthmatic patients than in non-asthmatic patients. Both asthmatic and non-asthmatics with pneumonia or bronchitis had higher concentrations of blood cytokines. |

| | | | | |
|-------------------------------|-------------------------|---|--|--|
| | Pharynx | Kama Y, et al. 2020 [109] | Pharyngeal samples of outpatients and/or in patients children with acute exacerbations of asthma (n= 111) (median age: 2.8/2.6, respectively) | The 3 major bacterial pathogens were <i>Streptococcus pneumoniae</i> (29.7%), <i>Moraxella catarrhalis</i> (11.7%), and <i>Haemophilus influenzae</i> (10.8%). Patients with <i>S. pneumoniae</i> colonization had significantly shorter wheezing episodes and reduced lung inflammation (including lower level of TNF- α) |
| Asthma natural history | Nasopharynx | Teo SM, et al. 2018 [110] | 244 infants with acute respiratory tract illnesses (ARIs) followed through their first five years of life | Dominance with <i>Moraxella</i> , <i>Haemophilus</i> and <i>Streptococcus</i> cluster were positively associated with acute respiratory tract illness. This change frequently preceded the detection of viral pathogens and acute symptoms. These changes were associated with ensuing development of persistent wheeze in children developing early allergic sensitization |
| | Nasal microbiota | Zhou Y, et al. 2019 [113] | A 1 year longitudinal study in school-age children with mild-moderate persistent asthma treated with daily ICS (mean age 8.0 \pm 1.8 years) | Children with nasal microbiota dominated by the commensal <i>Corynebacterium / Dolosigranulum</i> cluster at steady state experienced the lowest rates of exacerbation and disease progression. A switch towards the <i>Moraxella</i> - cluster was associated with highest risk of severe asthma exacerbation. |
| | Lung | Mansbach JM, et al. 2020 [111] | 842 infants hospitalized for bronchiolitis with a follow -up at 3 years | Increased abundance of <i>Moraxella</i> or <i>Streptococcus</i> species 3 weeks after day 1 of hospitalization was associated with an increased risk of recurrent wheezing. Increased <i>Streptococcus</i> species abundance the summer after hospitalization was also associated with a greater risk of recurrent wheezing |
| | Oropharynx | Cuthbertson L, et al. 2019 [112] | Oropharyngeal swabs were collected from 109 children hospitalized for acute wheezing / 75 non-wheezing controls. | No significant difference in bacterial diversity between wheezers and healthy controls. In wheezers, attendance at kindergarten or preschool was, associated with increased bacterial diversity.in contrast with rhinovirus (RV) infection |
| | Nasopharynx | McCauley K. et al. 2019 [35] | 312 school-age asthmatic patients enrolled in a trial of omalizumab, nasal secretion samples collected after randomization | Nasal microbiotas dominated by <i>Moraxella</i> species were associated with increased exacerbation risk and eosinophil activation. <i>Staphylococcus</i> or <i>Corynebacterium</i> species–dominated microbiotas were associated with reduced respiratory illness and exacerbation events. <i>Streptococcus</i> species–dominated microbiota increased the risk of RV. |
| | Sputum | Abdel-Aziz MI. et al. 2020 [114] | Sputum samples were collected in 100 severe asthmatics of the U-BIOPRED adult patients cohort at baseline and after 12-18 months of follow-up | Two microbiome-driven clusters were identified, characterized by asthma onset, smoking status, treatment, lung spirometry results, percentage of neutrophils and macrophages in sputum. Patients of the most severe cluster displayed a commensal-deficient bacterial profile which was associated with worse asthma outcomes. Longitudinal clusters revealed high relative stability after 12-18 months in the severe asthmatics. |
| | Lung (BAL) | Robinson PFM, et al. 2019 [34] | Children: 35 wheezers categorized as MTW or EVW BAL obtained at steady state | There was no relationship between lower airway inflammation or infection and clinical preschool wheeze phenotypes. 2 groups identified: 1) a <i>Moraxella</i> species dysbiotic microbiota cluster that associated with airway neutrophilia and a mixed microbiota; 2) a mixed microbiota cluster with a macrophage- and lymphocyte-predominant inflammatory profile |

2

3 **Table 2: Major human studies (pediatric and adult population) assessing the impact of pathogenic bacteria and microbiota during the**
4 **development, the attack and the natural history of pediatric asthma.**

5 BAL: Broncho-alveolar lavage; EVW: Episodic Viral Wheeze, MTW: Multiple trigger Wheeze, RSV: Respiratory syncytial virus; RV: Rhinovirus

Graphical Abstract

- Characteristics of host-microbiota interactions in asthmatic children favor asthma attacks and may allow to define asthma endotypes.
- This knowledge offers promising perspectives to treat asthmatic children using precision medicine

