1   Evolving Concepts in how Viruses Impact Asthma
2
3   Matthew C. Altman, M.D.
4   Department of Medicine
5   University of Washington
6   Seattle, Washington
7
8   Avraham Beigelman, M.D.
9   Division of Pediatric Allergy, Immunology & Pulmonary Medicine
10   Washington University School of Medicine
11   St. Louis MO
12   The Kipper Institute of Allergy and Immunology
13   Schneider Children's Medical Center of Israel
14   Petach Tikvah, Israel
15
16   Christina Ciaccio, M.D., M.Sc.
17   Allergy/Immunology and Pediatric Pulmonology and Sleep Medicine
18   University of Chicago School of Medicine
19   Chicago, IL
20
21   James E. Gern, M.D.
22   Division of Pediatric Allergy, Immunology and Rheumatology
23   Department of Pediatrics
24   University of Wisconsin School of Medicine and Public Health
25   Madison, WI
26
27   Peter W. Heymann, M.D.
28   Department of Pediatrics
29   University of Virginia Medical Center
30   Charlottesville, VA
31
32   Daniel J. Jackson, M.D.
33   Division of Pediatric Allergy, Immunology and Rheumatology
34   Department of Pediatrics
35   University of Wisconsin School of Medicine and Public Health
36   Madison, WI
37
38   Joshua L. Kennedy, M.D.
39   Division of Allergy/Immunology
40   University of Arkansas for Medical Sciences
41   Little Rock, AR
42
43   Kirsten Kloepfer, M.D., M.S.
44   Division of Pediatric Pulmonology, Allergy and Sleep Medicine
45   Department of Pediatrics
46   Indiana University School of Medicine
47   Indianapolis, IN
48
49   Robert F. Lemanske, Jr., M.D.
50   Division of Pediatric Allergy, Immunology and Rheumatology
51   Department of Pediatrics

_____

52   University of Wisconsin School of Medicine and Public Health
53   Madison, WI
54
55   Laurie M. McWilliams, M.D.
56   Allergy Partners of the Triangle
57   Raleigh, N.C.
58
59   Lyndsey Muehling, Ph.D.
60   Department of Medicine
61   University of Virginia Medical Center
62   Charlottesville, VA
63
64   Christy Nance, Ph.D.
65   Departments of Pediatrics and Immunology/Pathology
66   Baylor College of Medicine
67   Houston, TX
68
69   R. Stokes Peebles, Jr., M.D.
70   Division of Allergy, Pulmonary, and Critical Care Medicine
71   Vanderbilt University Medical Center
72   Nashville, TN
73
74
75   Address correspondence to: Stokes Peebles, M.D.
76   Division of Allergy, Pulmonary and Critical Care Medicine
77   Vanderbilt University School of Medicine
78   T-1218 MCN, VUMC
79   1161 21st Ave South
80   Nashville, TN 37232-2650, USA
81   Telephone: 615-343-3412; fax: 615-343-7448
82   E-mail: stokes.peebles@vanderbilt.edu
83
84

86

Abstract

Over the last decade, there have been substantial advances in our understanding about how viral infections regulate asthma (Table 1). Important lessons have been learned from birth cohort studies examining viral infections and subsequent asthma, understanding the relationships between host genetics and viral infections, the contributions of respiratory viral infections to patterns of immune development, the impact of environmental exposure on severity of viral infections, and how the viral genome influences host immune responses to viral infections. Further, there has been major progress in our knowledge about how bacteria regulate host immune responses in asthma pathogenesis. In this article, we also examine the dynamics of respiratory tract bacterial colonization during viral upper respiratory tract infection, in addition to the relationship of the gut and respiratory microbiomes with respiratory viral infections. Finally, we focus on potential interventions that could decrease virus-induced wheezing and asthma. There are emerging therapeutic options to decrease severity of wheezing exacerbations caused by respiratory viral infections. Primary prevention is a major goal and a strategy toward this end is considered.

Abbreviations:
Respiratory syncytial virus (RSV)
Rhinovirus (RV)
Airway responsiveness (AR)
Fusion (F)
Type 2 (Th2)
Cadherin-related family member 3 (*CDHR3*)
Genome wide association study (GWAS)
Environmental tobacco smoke (ETS)
Upper respiratory tract infection (URI)
Short chain fatty acids (SCFA)
Forced expiratory volume in 0.5 s ($FEV_{0.5}$)
Wheezing lower respiratory tract illness (WLRI)
Inhaled corticosteroids (ICS)
Long acting beta agonists (LABA)
Lower respiratory tract illness (LRTI)
Plasmacytoid dendritic cells (pDC)

123
124 **Introduction**

125

126 Over the last decade, there have been substantial advances in our understanding about how

127 viral infections regulate asthma (Table 1). Important lessons have been learned from birth

128 cohort studies examining viral infections and subsequent asthma, understanding the

129 relationships between host genetics and viral infections, the contributions of respiratory viral

130 infections to patterns of immune development, the impact of environmental exposure on severity

131 of viral infections, and how the viral genome influences host immune responses to viral

132 infections. Further, there has been major progress in our knowledge about how bacteria

133 regulate host immune responses in asthma pathogenesis. In this article, we also examine the

134 dynamics of respiratory tract bacterial colonization during viral upper respiratory tract infection,

135 in addition to the relationship of the gut and respiratory microbiomes with respiratory viral

136 infections. Finally, we focus on potential interventions that could decrease virus-induced

137 wheezing and asthma. There are emerging therapeutic options to decrease severity of

138 wheezing exacerbations caused by respiratory viral infections. Primary prevention is a major

139 goal and a strategy toward this end is considered.

140

The viral genome and how it influences host immune responses to viral infections

Respiratory syncytial virus (RSV) and rhinovirus (RV) are important causes of wheezing in early life and wheezing illness with these viruses have been associated with increased asthma risk later in childhood. At age 6, there is an increased risk of asthma if children had wheezing illness with RSV (odds ratio 2.6), RV (odds ratio 9.8), or both RSV and RV (odds ratio 10.0) in the first 3 years of life. RSV is a negative-sense, single stranded RNA virus that is a member of the Paramyxoviridae family and is the leading cause of hospitalization each year in the United States in children under 1 year of age.[1] There are three species of RV in the enterovirus genus, and all are positive-sense, single-stranded RNA viruses that have protein capsids. RV are the most frequently detected viruses in wheezing children over the age of 1 year, and from children and adults with acute exacerbations of asthma.

The clinical manifestations of a viral infection in the respiratory tract result from a complex interplay of the host, environment, and virus. To make comparisons between different immune responses elicited by diverse viruses, host and the environmental conditions must be held constant in order to prevent the introduction of confounding factors. This requires artificial conditions, such as the use of human cell lines for *in vitro* infection studies, the infection of genetically identical animals, such as mice, housed in the same environment, and the use of a standard viral inoculum. Determining the effect of specific genes within a virus requires that all other viral genes are identical. Such studies have begun but are still relatively new.

Experiments in models of RSV genomes have provided important insights into how the viral genome influences host immune responses to infection. Three RSV strains commonly used in pathogenesis studies are A2, line 19, and Long. RSV A2 infection in BALB/c mice resulted in a predominant IFN-γ immune response, no production of the Th2 cytokine IL-13 in the lung, an absence of airway mucus, and no airway responsiveness (AR) to methacholine.[2] Infection with RSV Long similarly did not result in host IL-13 production in the lung nor was there airway mucus.[3] However, line 19 infection in genetically identical mice in the same environment

167   caused the host immune response to produce IL-13, decreased IFN-γ compared to A2 infection,

168   airway mucus, and heightened AR.[2]   Sequencing of the A2, line 19, RSV Long strains revealed

169   six amino acid differences between line 19 and the A2 and Long strains, of which 5 amino acid

170   differences were in the fusion (F) protein.[3] To determine the contribution of the F gene of each

171   virus to disease pathogenesis, a reverse genetics approach was undertaken by creating

172   chimeric viruses whereby an A2 virus was manipulated to replace the A2 F gene with either the

173   F gene of either line 19 or Long. Infection with the chimeric virus containing the line 19 F gene

174   caused decreased host IFN-α lung levels, higher viral load in the lungs, greater lung IL-13

175   protein, augmented airway mucus, and increased AR, compared to the chimeric viruses

176   containing either A2 or Long F proteins.[3] Therefore, this reverse genetic approach provided the

177   opportunity to not only discover which genes in RSV line 19 strain were responsible for the lung

178   IL-13, airway mucus, and AR, but also the identification of the specific amino acids that caused

179   airway remodeling. These techniques not only provide the knowledge of unique components of

180   the viral genome that contribute to specific pathogenic features but may also assist in vaccine

181   and therapeutic strategies aimed at the proteins responsible for specific disease characteristics.

182   <u>Future perspectives</u>: To date, there have not been studies that reveal a relationship between

183   RSV genotypes and the presence of wheezing in hospitalized children with bronchiolitis or

184   bronchopneumonia; however, this may be a function of the lack of application of technology to

185   sequence strains because of cost. Studies relating viral genetics to severity of illness in mice

186   have demonstrated the intricate interactions between viral genome, viral proteins, host cell

187   function and metabolism and immune response. Developing a greater understanding of this

188   chain of events could highlight several therapeutic opportunities, including identification of high-

189   priority pathogens, inhibition of viral or host proteins that are critical for replication, and

190   strategies to inhibit virus-induced skewing of immune responses that favors viral replication over

191   host defense.

192

Host genetics and viral respiratory infections

193

194   A number of studies have begun to shed light on the relationships among host genetics,

195   viral infections and acute and long-term respiratory outcomes. Candidate gene approaches

196   have been utilized to identify associations between genetic polymorphisms and viral respiratory

197   illness outcomes. Polymorphisms in several antiviral and innate immune genes have been

198   linked to susceptibility to respiratory viruses, infection severity, and virus-induced asthma

199   exacerbations, and have been replicated across multiple cohorts (Table 2). These genes

200   include *STAT4, JAK2, MX1, VDR, DDX58, and EIF2AK2*.[4] Additionally, whole exome

201   sequencing has been utilized to identify rare variants in innate immune responses linked to

202   severe respiratory viral infections. Autosomal recessive *IRF7* deficiency has been observed in

203   one patient in association with severe influenza infection and acts through impairment of

204   interferon amplification.[5] Dominant negative loss-of-function variants in *IFIH1*, critical to viral

205   RNA sensing, have been shown to be a risk for intensive care unit hospitalization due to viral

206   infections in previously healthy children.[6]

207   A number of polymorphisms have been specifically associated with increased severity of

208   illnesses associated with hospitalization from RSV infection. A candidate gene approach

209   identified SNPs in the innate immune genes *VDR, IFNA5, and NOS2* as risk factors for RSV

210   bronchiolitis.[7] In order to further elucidate associations of host genetics with RSV illness severity

211   and asthma risk, a recent review examined overlap amongst genes associated with both

212   outcomes. This approach identified a number of genes involved with both innate immunity and

213   type 2 (Th2) inflammatory responses (*ADAM33, IL4R, CD14, TNF, IL13 and IL1RL1)* that are

214   highly relevant to these outcomes.[8]

215   The most replicated association between host genetics and asthma risk is the 17q21

216   locus. In two birth cohort studies, variants in this locus, including ORMDL3 and GSDMB, were

217   also associated with increased risk of wheezing with RV infections in early life.[9] Interestingly,

218   these variants were only associated with increased risk of subsequent asthma in children who

219   developed RV wheezing in the first 3 years of life. In contrast, early life RSV wheezing was not

220   linked to 17q21 variants in these cohorts. In addition, farm exposures[10] and pets[11] in the home

221   lessen the risk of asthma for children with high-risk 17q21 genotypes. In each case, the genetic

222   risk associated with 17q21 was buffered by protective environmental exposures.

223   RV virulence varies by species; RV-A and RV-C are more likely to cause illnesses,

224   wheezing and lower respiratory tract infection compared to RV-B,[12] which has a slower rate of

225   replication and induces muted cytokine and chemokine responses.[13] Whether there are

226   individual types within RV species that are more virulent is unknown, and difficult to study given

227   the genetic diversity of these viruses and high mutation rate. A functional polymorphism in

228   cadherin-related family member 3 (*CDHR3*) has been associated by genome wide association

229   study (GWAS) with early childhood asthma and severe wheezing episodes.[14] Interestingly,

230   CDHR3 is a receptor that enables binding and replication of RV-C, suggesting that this link

231   between *CDHR3* and asthma risk may be mediated by RV-C infections.[15] In support of this

232   hypothesis, children with the risk polymorphism in *CDHR3* were recently found to have greater

233   risk of RV-C illnesses, but not illnesses associated with other viruses.[16]

234   <u>Future perspectives:</u> These genetic associations among respiratory virus susceptibility, infection

235   severity and subsequent asthma risk may prove to be important to risk stratify populations, and

236   potentially provide new therapeutic targets for reducing illness severity and subsequent risk.

237   Further, unbiased approaches have been employed recently to identify pathways of gene

238   expression in the upper airway that differentiate a viral cold that resolves from one that leads to

239   an asthma exacerbation.[1] Efforts are ongoing to understand how these gene expression

240   patterns are regulated in hopes of identifying new personalized therapeutic strategies to prevent

241   asthma exacerbation. The integration of multiple "omics" approaches holds promise to provide

242   the ability to unravel these complex relationships.

243

244   **Environmental factors affecting the inception and severity of asthma exacerbations**

245   The exposome, defined as the measure of all exposures that influence the health of an

246   individual, is an important determinant of asthma risk during the lifespan of an individual.[17] Early

247   exposures can set in motion pathways that will ultimately define illnesses and symptom

248   exacerbations, which is especially true when considering the ontogeny of asthma. From birth

249   through school years, children are frequently exposed to a variety of respiratory pathogens,

250   allergens, microbes and airway irritants. The pathogenic or beneficial effects of these

251   exposures and their interactions remain the focus of research to develop new interventions and

252   preventive therapies.

253   Most of the initial research devoted to the ontogeny of asthma focused on RSV infections

254   which are frequently detected by culture and tests for RSV antigen in nasal washes from

255   wheezing infants during the mid-winter months. Studies in the past reported that flares of

256   wheezing caused by RSV leading to hospitalization during infancy increased the risk for

257   developing asthma and allergy.[18, 19] However, recent studies indicate that the more severe

258   episodes (i.e., those requiring hospitalization) of infantile wheezing caused by RSV increase the

259   risk for subsequent wheeze in infants and toddlers, but it is less certain whether RSV-induced

260   wheeze influences the development of atopy or asthma as children grow older.[20]

261   In contrast, flares of wheezing caused by RV are more strongly linked to persistent

262   wheezing and the development of asthma, especially in children who are sensitized to allergens

263   at an early age.[21-23] In keeping with this, the dominant risk factors for asthma attacks that require

264   hospital care among children after 3 years of age is the combination of allergic airway

265   inflammation and RV infection.[24-26] As a result, several host factors should be considered in

266   efforts to treat asthma exacerbations more effectively and to reduce the risk for asthma

267   development, For example:

268   1) There may be phenotypes of asthmatic children who would benefit from development of

269   vaccines to RV or RSV. For example, genetic variations at the 17q21 locus and a coding

270   SNP in *CDHR3* (the receptor for RV-C genotypes) increase the risk for wheezing with

271   RV during childhood.[9, 14]

272   2) There is current interest in whether the administration of a biologic such as omalizumab

273   (anti-IgE antibody) during early childhood will have a disease modifying effect after this

274   intervention is discontinued (i.e., the Preventing Asthma in High Risk Kids (PARK) trial;

275   NCT02570984).

276   3) There is evidence that the asthmatic airway, especially epithelial cells and innate

277   lymphoid cells, has a Type 2 bias with enhanced production of TSLP, IL-13, and IL-25 in

278   parallel with decreased type I and III IFN responses that are needed for effective anti-

279   viral killing and clearance.[27-34] This bias may increase the susceptibility of allergic

280   asthmatics to RV infections. Once infected, however, *in vivo* studies have shown that

281   during RV infections viral loads and clearance are similar among children and young

282   adults with asthma compared to non-allergic individuals without asthma.[31] At present,

283   mechanisms to explain these differences are poorly understood. A better understanding

284   is likely to come from research focused on the cascade of early, innate cellular and

285   molecular events that follow RV infection of epithelial cells.

286   Future perspectives: Airway inflammation caused by recurrent infections (predominantly with

287   RV) in the allergic host will continue to be the focus of research designed to develop new

288   therapies to help children and young adults with asthma. Whether treatments targeting allergic

289   inflammation will be sufficient to reduce the frequency and severity of exacerbations (e.g., using

290   new monoclonal antibody-based biologics), or whether additional therapeutics will be needed to

291   decrease the frequency of RV infections, or enhance viral killing, remains to be determined.

292   Looking to the future, the evaluation of other interventions such as the administration of

293   antibiotics to treat secondary bacterial pathogens, or azithromycin to reduce wheezing following

294   virus infection also deserve further study, along with investigations to determine whether the

295   administration of vitamin D, probiotics, and dietary modifications (e.g., fish oil) will have benefits.

296   In contrast, the adverse effects of airway irritants such as environmental tobacco smoke (ETS)

297   and air pollution (e.g., diesel fuel) on the severity and persistence of RV-induced asthma remain

298   poorly understood.

299

300   **Effects of respiratory viral infections on patterns of immune development**

301   Acute wheezing illnesses with respiratory tract viruses in infancy and early childhood

302   represent an important risk factor for childhood recurrent wheezing and later asthma

303   development. This link is particularly well-established with RV and RSV, suggesting that these

304   viruses may have a causative role, and significant research is directed towards understanding

305   how these viruses can alter immune development to contribute to asthma pathogenesis. That

306   said, causation remains unproven and asthma prevention strategies targeting viral illnesses do

307   not currently exist.

308   RV-associated wheezing, in particular, is associated with a higher asthma risk than other

309   viruses; this has been consistently demonstrated across multiple studies.[21, 23, 35-38] Many of

310   these studies have linked RV-induced wheezing with other asthma risk factors, in particular

311   markers of atopy including allergic sensitization, increased eosinophils, and atopic eczema,

312   suggesting possible additive or synergistic effects in increasing asthma risk. Experimental

313   models have demonstrated alterations in type-2 immune responses to RV that may account for

314   this risk (Figure 1). Mouse models have demonstrated that neonatal RV (RV1B) infection results

315   in persistent airway hyperresponsiveness, mucous cell metaplasia, and IL-13 production that

316   does not occur in adult mice. Furthermore, knockout of IL4R prevents this response, consistent

317   with an IL13-dependent process.[39] Subsequent work demonstrated that RV infection leads to

318   expression of epithelial derived cytokines IL-25, IL-33, and TSLP and an increase in ILC2s as

319   an important source of airway IL-13; blocking these pathways with anti-IL-25 attenuates

320   neonatal RV-induced AHR and mucous cell metaplasia.[40, 41] While there is no equivalent human

321   evidence regarding the immune effects of RV in early life, these same pathways are known to

322   play a key role in the response to RV leading to exacerbations in established asthma.[29]

323   A key question however, is whether underlying Th2 inflammation or RV associated

324   wheezing comes first. A prospective birth cohort analysis has shown that allergic sensitization

325   generally precedes RV wheezing but not the other way around, suggesting allergic sensitization

326   may lead to more severe RV illnesses and the development of asthma.[42] Supporting this

327   observation, *in vitro* studies have shown that Th2 inflammation can inhibit type I and III

328   interferon antiviral responses to RV infections,[43, 44] which may increase susceptibility to more

329   severe RV infections. However in contrast, several human studies have demonstrated

330   increased IFN signatures in asthmatic children with virus infections, as well as in severe asthma

331   in adults;[45-47] these might represent different disease states, as a recent report found that early

332   life exacerbation-prone asthma was correlated with low IFN signatures, while the highest IFN

333   signatures were associated with later-onset asthma.[48]

334   Allergy is a major risk factor for the progression from wheezing illnesses to asthma, and

335   this has been a very consistent finding across multiple cohorts.[21, 49, 50] Allergic sensitization

336   precedes wheezing illnesses in most young children,[42] and allergic inflammation can impair

337   antiviral responses *in vitro*[51] and *in vivo*.[52] This suggests that allergic airway inflammation can

338   increase susceptibility to and severity of viral respiratory illnesses. Allergic sensitization in early

339   childhood may also modify the relationship between microbial colonization and respiratory

340   outcomes. In preschool children whose airways were colonized with pathogen-dominated

341   microbiomes, sensitized children were at increased risk for chronic asthma while non-sensitized

342   children were likely to have transient wheeze that resolved by age 4 years.[53]

343   It is well established that hospitalization for RSV bronchiolitis in the first year of life is

344   associated with later development of asthma.[38, 54-56] RSV induces a broad innate immune

345   response in infants including systemic interferon, neutrophil, and inflammatory pathways, and

346   distinct RSV strains and concomitant airway bacteria can influence the severity of infection.[57, 58]

347   The risk for more severe RSV-illnesses has also been linked to polymorphisms in several

348   immune regulatory genes,[59] many of which also can influence asthma risk. However, whether

349   RSV is causal remains a subject of debate with two large cohort studies showing different

350   conclusions,[59, 60] one suggesting causation and the other an underlying genetic predisposition.

351   Notably, two prevention studies using palivizumab (a monoclonal antibody directed against

352   RSV) in high-risk infants found that prevention of more severe RSV-illnesses decreased the risk

353   of childhood recurrent wheezing but not asthma development.[61, 62] Ultimately RSV infection

354   appears to have the greatest impact on asthma risk during a critical window of lung

355   development for infants born during the fall (in the Northern hemisphere) who are at ~4 months

356   of age during the peak of the winter RSV season. It has been well-established that RSV

357   infection can induce pathologic Th2 immune responses, especially within the context of

358   formalin-inactivated RSV vaccination.[63-65] More recently, studies in mice have demonstrated the

359   ability for RSV-related Pneumonia Virus of Mice as well as human RSV infection to break

360   tolerance to allergens in neonatal mice.[66] Furthermore, it is now appreciated that RSV triggers

361   release of epithelial-derived cytokines that promote Th2 responses and can induce ILC2

362   responses following infection.[67, 68] These same epithelial cytokines have also been implicated in

363   RV infection, perhaps suggesting a shared innate Th2-skewing mechanism during viral

364   infection.[27, 40]

365   Future perspectives: Fully understanding the patterns of immune development that lead to

366   asthma inception, and how such patterns are affected by exogeneous exposures including viral

367   infections, will direct asthma prevention research. Ongoing studies are focused on altering Th2-

368   skewing in early life including through blocking IgE and through altering microbial exposures. If

369   effective, decreasing Th2-inflammation in early life may function in part through enhancing

370   antiviral responses.[51, 69] However, antiviral specific therapies including RV and RSV vaccines,

371   may also prove to be critical in asthma prevention.

372

373 **Dynamics of respiratory tract bacterial colonization during viral upper respiratory tract**

374 **infection**

375   Detection of viruses in the upper airway during peak viral seasons can be as high as

376 90% in prospective studies.[70] However, rates of illness are significantly lower, leading

377 researchers to question why some patients are more susceptible to increased morbidity when

378 they have a viral upper respiratory tract infection (URI). One factor that has been shown to

379 increase upper and lower airway symptoms during viral infections are bacteria.[71]

380   These bacteria collectively constitute the microbiota. The upper airway microbiota

381 develops over the first year of life with alterations in the natural development associated with

382 increased risk for URIs during the first few years of life.[72, 73] The most abundant bacteria within

383 the upper airway of infants and children are *Staphylococcus*, *Streptococcus*, *Moraxella*,

384 *Haemophilus*, *Dolosigranulum*, and *Corynebacterium*.[71, 72, 74-78]

385   In several infant cross-sectional and cohort studies, the presence of *Streptococcus*,

386 *Moraxella*, or *Haemophilus* during upper respiratory infection increases the likelihood that the

387 infant will have lower airway symptoms.[72, 76] Studies examining airway bacteria during RSV

388 bronchiolitis have reported links between an increased abundance of *Streptococcus*[9] and

389 *Haemophilus*.[78] In contrast, RV-bronchiolitis is associated with an increased abundance of

390 *Moraxella* and *Haemophilus*.[9] While these studies suggest that a bacteria-virus interaction

391 occurs during infancy, only a few studies have examined the association between virus and

392 bacteria in school-age children. One such study revealed that children with *Streptococcus* or

393 *Moraxella* present in their airway are more likely to have cold and asthma symptoms during a

394 naturally occurring RV infection.[71] Collectively, these studies demonstrate that an association

395 exists between specific bacteria and illness severity.

396   While *Streptococcus*, *Moraxella* and *Haemophilus* are often associated with an increase

397 in viral-associated symptoms, a higher abundance of *Corynebacterium*, *Staphylococcus* and

398 *Dolosigranulum* is often present in the airway in the absence of viral detection and clinical

399 symptoms.[75, 77, 78] In addition, when the latter three bacteria are enriched in the upper airway,

400 infants are less likely to have an acute respiratory illness,[72] and school-age children are less

401 likely to have a symptomatic illness during RV infection.[75] Furthermore, high abundance of

402 *Lactobacillus* in the upper airway during RSV illness is associated with a decreased risk of

403 childhood wheeze,[77] suggesting that bacteria present in the airway during viral illnesses may

404 contribute to both illness severity and long term sequela.

405 Future perspectives: Because most studies examining airway bacteria during viral infection

406 have been cross-sectional, observational studies, it remains unclear how airway microbes affect

407 the epithelium, and whether these interactions contribute to the causation of wheezing illnesses,

408 asthma development in young children, and exacerbations of established asthma. Greater

409 insight is needed into metabolic, immunologic and toxic effects of bacteria on epithelial cells that

410 could contribute to acute illnesses and asthma risk. While many studies have examined

411 changes in bacteria that occur during viral infection, few have examined how the airway

412 microbiome influences susceptibility vs. resilience to viral infection. Some bacteria could

413 promote a "pro-inflammatory" environment thereby making the airway susceptible to viral

414 infection. The presence of *H. influenzae* in the infant airway prior to viral infection is associated

415 with increased expression of local inflammatory cytokines suggesting a link between bacteria

416 and airway inflammation.[79] In contrast, mice receiving intranasal administration of *Lactobacillus*

417 *rhamnosus* prior to viral infection have enhanced antiviral immune responses,[80] suggesting that

418 some bacteria protect the airway and reduce the risk of symptomatic viral infection. Greater

419 understanding of these relationships may lead to new preventive approaches to acute viral-

420 bacterial illnesses and perhaps the development of childhood asthma.

421

422 **The influence of the gut microbiome on viral infections of the respiratory tract**

423   The gut microbiome represents the most abundant and diverse microbial environment in

424 the human body, comprised of approximately 40 trillion bacteria.[81] These bacteria have

425 coevolved with humans over millennia to contribute to a symbiosis in which humans consume

426 prebiotic fiber which is metabolized by resident microbes in the gut to create short chain fatty

427 acids (SCFA) which in turn regulate immune responses.[82, 83] Alterations in this relationship are

428 occurring in modern times due to practices such as the frequent use of antibiotics, and the

429 consumption of a high-sugar, low fiber diet. As a consequence, a state of microbial dysbiosis,

430 or an ecological imbalance, may result, which leads to the loss of metabolic capabilities and

431 predisposes infants to both the development of atopic diseases as well as an increased

432 susceptibility to viral infections.[84]

433   Although epidemiologic evidence strongly supports a role of the gut microbiome in the

434 development of asthma, the mechanisms remain unclear.[85, 86]   The most popular theory to

435 explain these observations is that colonization with certain gut bacteria have a direct anti-

436 inflammatory effect on the respiratory tract decreasing the likelihood of airway hyperreactivity.[87]

437 However, there is evidence that certain species of microbiota in the gastrointestinal tract prime

438 the respiratory immune system to effectively fight viral pathogens. Immunologic factors in early

439 life such as low blood cell interferon responses[88-91] and attenuated cytokine production[92] have

440 been associated with increased risk for wheezing in infancy. Furthermore, patterns of

441 metabolites (which can regulate immune responses[93]) at birth are associated with the risk for

442 wheezing illnesses.[94] The idea that delayed immune maturation might contribute to wheezing is

443 supported by studies showing that early life exposures to dogs,[95, 96] farm life,[10, 97] and increased

444 microbes and allergens[98] are inversely related to the risk of wheezing illnesses. Furthermore,

445 exposure to these microbes and allergens during the prenatal period or infancy may be

446 immunostimulatory.[99, 100] A loss, therefore, of these resident microbes may then lead to a

447 predisposition to viral infections and in turn, the development of asthma.

448   Several studies have proposed mechanisms for the influence of the gut microbiota on

449 both local and distant immune functions. SCFA have been shown to have a local effect on

450 immune responses through their influence on mucosal barrier function, and a loss of SCFA-

451 producing bacteria has been implicated in the development of food allergy.[101] Recent advances

452 have also shown that this symbiosis also influences vital immune responses in other systemic

453 tissues. For example, in the absence of SCFA, mucosal barrier function can break down and

454 allow for translocation of gut pathobionts, bacteria that are symbiotic under normal conditions

455 but pathogenic when removed from their normal environment, which, in turn, can drive

456 autoimmunity.[102] Similarly, in a murine model intact commensal bacteria in the gut were

457 required for adaptive immune responses to respiratory influenza virus infection. Specifically,

458 when mice were treated with antibiotics, they had reduced virus-specific antibody titers, CD4+

459 T-cell responses, and cytokine secretion which consequently resulted in elevated viral titers post

460 infection. This impairment, however, was rescued by local or distal injection of Toll-like receptor

461 ligands.[103] Further, exposure to house dust from homes with dogs enriched the cecal

462 microbiome in a murine model with *L. johnsonii*, which protected them against infection with

463 RSV.[104]

464 Future Perspectives: Although the pathways remain incomplete, evidence continues to mount

465 that the gut microbiome can influence the maturation of the immune system in viral defense and

466 therefore the development of asthma.[105] Future therapies look to a role of probiotics for the

467 prevention and treatment of allergic disorders, with recent evidence that atopy risk may be

468 associated with a dysbiosis of the gut microbiome. Studies have shown that in asthma, MMP9

469 (members of a family of enzymes that cleave extracellular matrix proteins) levels were

470 significantly increased and treatment with the probiotic, *L. rhamnosus* GG (LCC), decreased

471 MMP9 expression in lung tissue and inhibited inflammatory cell infiltration, as well as reducing

472 exhaled nitric oxide among 4- to 7-year olds in pediatric asthma.[106]

473 In early childhood, total fecal IgE levels appear to be specifically correlated with house dust

474 mite-specific IgE levels, indicating that fecal IgE levels represent markers of allergic response to

475 aeroallergens. A significant correlation of fecal IgE levels with *Dorea spp.* and *Clostridium spp.*

476 related to allergic rhinitis and asthma, respectively, suggest that modulation of particular subsets

477   of gut microbial dysbiosis could contribute to the susceptibility to allergic airway diseases.[107]

478   Future work is required for identification of specific species and functional studies to understand

479   the strength and mechanism of these associations. In the future, it is critical to understand more

480   precisely the microbiota composition. Optimized biomarker studies of the microbial taxa and the

481   metabolites involved in asthma-associated dysbiosis could help identify infants at risk of asthma

482   before symptoms. This would also provide a scientific rationale for future therapeutic strategies

483   aimed at restoring an altered infant gut microbiome. Future studies need to revolve around

484   state-of-the-art methods for the evaluation of the microflora to better define indications, the

485   probiotic strains and the type of prebiotic to be used.

486

487   **Potential for primary prevention: clinical trials aiming to prevent the development of the**

488   **episodic wheeze phenotype**

489   The inception of childhood asthma is tightly related to early life events such as

490   respiratory infections and the development of aeroallergens sensitization. Other co-factors (e.g.,

491   vitamin D) may modulate asthma inception pathways. Previous and on-going clinical trials,

492   geared for asthma prevention, have targeted these pathways and co-factors.

493   Early life respiratory infections are significant determinants of childhood asthma[108]. In

494   young toddlers, prevention of severe RSV bronchiolitis may reduce the risk of episodic

495   wheeze/asthma development [109, 110]. In preterm infants (33-35 wks), palivizumab treatment

496   during the RSV season resulted in a 73% reduction in the number of wheezing days during the

497   first year of life, and outside of the RSV season[109]. A follow up study from the same cohort,

498   revealed that at the age of 6 years the intervention resulted in a 41% relative risk reduction in

499   parent-reported asthma, but the forced expiratory volume in 0.5 s ($FEV_{0.5}$) percentage predicted

500   values, which was an additional primary outcome, were similar between the palivizumab and

501   placebo treated infants[110].

502   Since early life respiratory infections cannot be completely prevented, attenuation of the

503   immune/inflammatory processes during these infections may be another pathway for asthma

504   prevention. This concept is illustrated by the results of a proof-of concept clinical trial in 40

505   infants hospitalized with RSV bronchiolitis. In this trial, azithromycin treatment for 2 weeks,

506   during acute RSV bronchiolitis, reduced the likelihood of developing recurrent wheeze during

507   the subsequent year[111]. Azithromycin effects were attributed to anti-inflammatory properties

508   and/or its effects on the airway microbiome[112]. A larger confirmatory trial is ongoing (APW-RSV

509   II; NCT02911935; Table 3).

510   Based on observational studies that linked maternal vitamin D deficiency to childhood

511   asthma, two clinical trials (VDAART[113], COPSAC2010[114]) investigated whether maternal vitamin

512   D supplementation (2400 IU/day[113], 4000IU/day[114]) during pregnancy would prevent

513   asthma/recurrent wheeze in their children. A recent meta-analysis that combined these two

514   trials revealed that this intervention resulted in a 25% significant reduction in asthma/recurrent

515   wheeze risk during the first 3 years of life [115]. The effect was most profound among women with

516   sufficient serum vitamin D levels at randomization highlighting the importance of normal pre-

517   conception vitamin D levels[115]. It was suggested that vitamin D beneficial effects may be related

518   to enhancement of in-utero lung growth and development and promotion of antimicrobial effects,

519   thereby reducing early life respiratory infections, and/or providing immune modulation effects [116].

520   Omega-3 fatty acids were suggested to have anti-inflammatory effects, potentially due to

521   decreased production of arachidonic acid metabolites. In a recent clinical trial, high dose

522   Omega-3 fatty acids supplementation (2.4 g daily) to pregnant women, beginning at 24 week of

523   gestation, resulted in a 30% relative risk reduction of persistent wheeze or asthma at age 3

524   years[117]. These positive effects were driven by subgroups of children born to mothers with a

525   variant of the gene encoding fatty acid desaturase, predisposing to low ability to produce

526   omega-3 fatty acids, and by infants born to mothers with low omega-3 fatty acids baseline blood

527   levels. These sub-group analyses suggest the plausibility of a precision-medicine approach of

528 this potential future intervention. Nevertheless, it is important to assure that high dose omega-3

529 fatty acids does not possess any safety issues, before omega-3 fatty acids may be utilized for

530 asthma prevention.

531 The ongoing ORBEX clinical trial (NCT02148796) is attempting to modulate the infant

532 immune system by treating high-risk preschool children with Broncho-Vaxom® for 2 years to

533 prevent/delay the development of wheezing lower respiratory tract illness (WLRI) during a third

534 observation year. Broncho-Vaxom® contains bacterial lysates and was previously shown to

535 reduce the rate of respiratory infections[118]. Hence, it is postulated that prevention of early life

536 WLRI will prevent the development of the recurrent wheeze phenotype. Finally, the ongoing

537 PARK clinical trial (NCT02570984) is targeting the association between allergic sensitization

538 and asthma inception. PARK investigates whether treatment of high-risk preschool children with

539 Omalizumab for 2-years would prevent asthma development, and whether the treatment would

540 decrease asthma severity among infants who will develop asthma, during an additional 2-year

541 observation period.

542 Future perspectives: This is an exciting time for all involved in childhood asthma prevention:

543 recent clinical trials have shown the feasibility of asthma prevention, and multiple clinical trials

544 are ongoing toward this goal. In addition to targeting type 2 immune responses, new

545 interventions are needed to inhibit viral replication, either with specific inhibitors or strategies to

546 boost the development of global antiviral responses in the airways. Finally, studies in farming

547 environments strongly suggest that environmental exposure can lower the risk of viral

548 respiratory illness in addition to reducing allergy.[10, 97] Identifying relevant mechanisms is likely to

549 lead to new preventive approaches to virus-induced wheeze and asthma.

550

551 **New therapeutic options to decrease severity of asthma exacerbations caused by**

552 **respiratory viral infection**

553   Recent studies have focused on short-term increases in standard asthma therapy,

554   vitamin D supplementation, azithromycin and anti-IgE therapy. However, mixed efficacy results

555   limit the widespread application of many of these therapies in clinical practice.

556   Maintenance inhaled corticosteroids (ICS) are effective in reducing the risk of asthma

557   exacerbations and, when combined with inhaled long acting beta agonists (LABA), this

558   decreases the risk further. However, exacerbations continue to occur. Attempts to increase

559   dosing of inhaled steroid with early signs of loss of asthma control with viral infection, termed

560   the "yellow zone", to decrease exacerbation risk, have yielded mixed results. GINA guidelines

561   suggest increasing ICS at onset of symptoms as part of a self-management plan

562   (http://www.ginasthma.org). A Cochrane database review (including five studies in adults and

563   three studies in children) concluded that current evidence does not support increasing ICS in

564   mild to moderate asthma patients as part of a self-management plan to treat exacerbations.[119]

565   A clinical trial examined this question further in 254 children aged 5-11 with history of

566   mild to moderate persistent asthma with at least one previous exacerbation in the past year.[120]

567   Children were treated for 48 weeks with low dose inhaled steroid and assigned to either

568   continue this or quintuple the dose for 7 days at onset of loss of asthma control. There was no

569   significant difference in the rate of severe exacerbations in the groups. The total corticosteroid

570   exposure in the high dose group was 16% higher (including both inhaled corticosteroid use and

571   prednisone) and there was an effect on linear growth velocity between the high dose and low

572   dose group (-0.23 cm/year), suggesting potential risk without identifiable benefit of the therapy.

573   Vitamin D levels have been inversely associated with asthma severity, including

574   hospitalization for severe infections.[121] A large study aimed at optimizing low Vitamin D levels

575   through supplementation did not reduce rates of colds or treatment failures in adults with

576   asthma.[122, 123] In contrast, a meta-analysis of seven randomized trials demonstrated a significant

577   reduction in asthma exacerbations, with the effect seen only in patients with low vitamin D at

578 baseline.[124] There are ongoing studies in children with asthma examining the possible role of

579 vitamin D supplementation in preventing asthma exacerbations (Table 3).

580   Current guidelines do not recommend the use of antibiotic treatment for episodes of

581 asthma-like symptoms in children, yet they are commonly used. A randomized, double-blind,

582 placebo-controlled trial conducted in the US, evaluated the role of early administration of

583 azithromycin in prevention of progression to severe lower respiratory tract illness (LRTI)

584 symptoms.[125] Preschool children, age 12-71 months, with history of recurrent severe wheezing

585 in the setting of LRTI, were randomized to azithromycin 12 mg/kg for 5 days (307 patients) or

586 placebo (300 patients). The medications were to be started as soon as the children developed

587 signs or symptoms that typically preceded the development of a severe LRTI. The primary

588 outcome measure was the number of respiratory tract infections not progressing to a severe

589 LRTI. The azithromycin group experienced a lower risk of progression to a severe LRTI than the

590 placebo group.

591   In the COPSAC$_{2010}$ (Copenhagen Prospective Studies on Asthma in Childhood 2010

592 cohort), children (age 1-3) with recurrent asthma-like symptoms within this cohort were enrolled

593 in a study to assess the duration of episodes when treated with azithromycin.[126] With each

594 episode of 3 days of consecutive symptoms (wheeze, cough, dyspnea), children were

595 randomized to receive 10 mg/kg azithromycin or placebo for 3 days. Seventy-two children from

596 the recurrent asthma-like symptoms group had 158 episodes. The azithromycin treatment

597 shortened the days of symptoms, 3.4 days compared with 7.7 days after placebo,

598 corresponding to a calculated reduction in episode length of 63.3%. More improvement was

599 seen when the treatment was started earlier in the episode; however, treatment did not

600 significantly affect the time to next episode of troublesome lung symptoms in children.

601   With these episodes, a hypopharyngeal aspirate was collected and cultured for common

602 bacterial pathogens and a nasopharyngeal aspirate was collected for viral PCR. Overall, the

603 presence of any cultured pathogenic bacteria did not significantly alter the treatment effect

604   compared to episodes without bacteria present; however, azithromycin was more effective in

605   those whose culture grew *H. influenzae*. The treatment effect in these studies is promising;

606   however, resistance to these antibiotics and eliminating commensal microbes along with

607   pathogens are concerns with repeated treatment.

608   Birth cohort studies have shown allergic sensitization to be a risk factor for RV-induced

609   wheeze.[42] Additionally, in one prospective cohort study, the severity of RV-triggered asthma

610   exacerbation increased as the degree of allergen sensitization increased, with serum IgE levels

611   (total IgE and allergen specific IgE) increasing from baseline during the exacerbation.[127]

612   Persistence of asthma by age 13 was most strongly associated with wheezing illness with RV

613   and aeroallergen sensitization in early life[37] suggesting a role for both viral infection and allergic

614   sensitization in the development of asthma.

615   A possible mechanism for impaired response to viral infections in allergic asthmatics is a

616   decreased secretion of IFN in response to viral infection. Purified plasmacytoid dendritic cells

617   (pDC) from patients with allergic asthma were shown to secrete less IFN-α in response to

618   exposure with influenza A virus.[51] Increased FcεRIα expression and serum IgE levels were

619   inversely associated with IFN- α secretion. The increased susceptibility to viral wheeze in atopic

620   patients and impaired antiviral response in these patients suggests a role for possible

621   therapeutic intervention to decrease allergic inflammation with the goal of decreasing asthma

622   exacerbations in response to viral infection.

623   Omalizumab, a humanized monoclonal antibody that selectively binds to IgE, has

624   recently been studied as an add-on therapy to prevent fall asthma exacerbations in atopic

625   asthmatics in the Preventative Omalizumab or Step-Up Therapy for Fall Exacerbations

626   (PROSE) study.[128] The PROSE study included 478 children, age 6-17, with respiratory allergy

627   and asthma, randomized to either inhaled corticosteroid boost, add on omalizumab or placebo.

628   All patients had guidelines-based care in addition to the add-on treatment (ICS boost,

629   omalizumab or placebo). Treatment was begun 4-6 weeks before the participant's school start

630   day and ended 90 days after school start date.   Omalizumab treatment significantly decreased

631   the odds of having at least 1 exacerbation, whereas boosting ICS did not reduce risk.

632   Omalizumab increased IFN-α responses to RV *ex vivo*. Within the omalizumab group, greater

633   restoration of IFN-α responses were associated with fewer exacerbations. In this trial,

634   omalizumab was associated with a decreased frequency of RV illnesses, decreased duration of

635   RV infection as well as decreased frequency of overall respiratory illness, and reduced peak RV

636   shedding.[52] Omalizumab reduced expression of FcεRIα on the surface of pDC and this

637   reduction was associated with lower exacerbation rates and correlated with enhanced IFN-α

638   production, suggestion a possible mechanism for the interaction between allergic sensitization

639   and virus-induced asthma exacerbations.[69] However, the connection between the pDC type I

640   IFN production and asthma exacerbation will benefit from further study.

641   In an observational study following children with asthma presenting with an acute

642   asthma exacerbation triggered by RV, the use of omalizumab for at least 4 weeks prior to

643   presentation was associated with reduced severity of exacerbation compared with patients

644   primarily treated with ICS.[129]   This suggests a benefit in not only frequency and duration of

645   asthma exacerbation, but also severity of exacerbation.

646   Another possible mechanism for the interaction between allergic sensitization and virus-

647   induced asthma exacerbations is the presence of anti-viral IgE in response to infection. In RSV

648   infection in infants, RSV specific IgE was detected in nasopharyngeal secretions, with

649   significantly higher titers in subjects with wheezing.[130, 131] Correlation of the peak titers with

650   degree of hypoxia was also noted. Following known exposure to a specific laboratory strain,

651   RV-specific IgE could be detected in human sera.[132] While the IgE response to RV and RSV

652   are associated with infection, the role of IgE in the host response to these infections is not fully

653   understood. Given the decreased exacerbations with use of omalizumab, further investigation

654   into the role of anti-viral IgE is indicated.

655 <u>Future perspectives</u>: Given the morbidity of RSV and RV infections in patients with asthma, a

656 consistent and effective treatment approach is highly desirable. While studies have found

657 possible benefits to treatment with azithromycin and omalizumab, the widespread use of these

658 treatment approaches is not currently justified. Further characterization of risk in this patient

659 population and additional work to delineate the mechanisms by which these drugs are effective

660 may lead to selection of patients most appropriate for these therapies.

661

662

663 **Conclusion**

664

665 There have been important advances in our knowledge of the relationship between viruses and

666 asthma over the last decade. Advances in scientific methods have provided innovative

667 opportunities to examine host, environment, and viral interactions that either protect against or

668 increase vulnerability to asthma development and exacerbations. The exploration of the

669 contribution of the respiratory and gut microbiome to virally-induced asthma is in its infancy and

670 we suspect that over the next 5 years there will be major advances in this area. Finally, primary

671 prevention is a major goal to diminish the morbidity of virally-mediated wheezing, asthma and

672 exacerbations. Until primary prevention becomes a reality, clinical trials examining the impact of

673 established medications, as well as novel therapies, will be critical to diminish the impact of viral

674 infections on wheezing and asthma.

675

676　　　　　Bibliography
677
678　1.　　Stier MT, Peebles RS, Jr. Host and Viral Determinants of Respiratory Syncytial Virus-
679　　　　　induced Airway Mucus. Ann Am Thorac Soc 2018; 15:S205-S9.
680　2.　　Lukacs NW, Moore ML, Rudd BD, Berlin AA, Collins RD, Olson SJ, et al. Differential
681　　　　　immune responses and pulmonary pathophysiology are induced by two different strains
682　　　　　of respiratory syncytial virus. Am. J Pathol 2006; 169:977-86.
683　3.　　Moore ML, Chi MH, Luongo C, Lukacs NW, Polosukhin VV, Huckabee MM, et al. A
684　　　　　chimeric A2 strain of respiratory syncytial virus (RSV) with the fusion protein of RSV
685　　　　　strain line 19 exhibits enhanced viral load, mucus, and airway dysfunction. J Virol 2009;
686　　　　　83:4185-94.
687　4.　　Loisel DA, Du G, Ahluwalia TS, Tisler CJ, Evans MD, Myers RA, et al. Genetic associations
688　　　　　with viral respiratory illnesses and asthma control in children. Clin Exp Allergy 2016;
689　　　　　46:112-24.
690　5.　　Ciancanelli MJ, Huang SX, Luthra P, Garner H, Itan Y, Volpi S, et al. Infectious disease.
691　　　　　Life-threatening influenza and impaired interferon amplification in human IRF7
692　　　　　deficiency. Science 2015; 348:448-53.
693　6.　　Asgari S, Schlapbach LJ, Anchisi S, Hammer C, Bartha I, Junier T, et al. Severe viral
694　　　　　respiratory infections in children with IFIH1 loss-of-function mutations. Proc Natl Acad
695　　　　　Sci U S A 2017; 114:8342-7.
696　7.　　Janssen R, Bont L, Siezen CL, Hodemaekers HM, Ermers MJ, Doornbos G, et al. Genetic
697　　　　　susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with
698　　　　　innate immune genes. J Infect Dis 2007; 196:826-34.
699　8.　　Larkin EK, Hartert TV. Genes associated with RSV lower respiratory tract infection and
700　　　　　asthma: the application of genetic epidemiological methods to understand causality.
701　　　　　Future Virol 2015; 10:883-97.
702　9.　　Caliskan M, Bochkov YA, Kreiner-Moller E, Bonnelykke K, Stein MM, Du G, et al.
703　　　　　Rhinovirus wheezing illness and genetic risk of childhood onset asthma. N Engl J Med
704　　　　　2013; 368:1398-407.
705　10.　　Loss GJ, Depner M, Hose AJ, Genuneit J, Karvonen AM, Hyvarinen A, et al. The Early
706　　　　　Development of Wheeze. Environmental Determinants and Genetic Susceptibility at
707　　　　　17q21. Am J Respir Crit Care Med 2016; 193:889-97.
708　11.　　Stokholm J, Chawes BL, Vissing N, Bonnelykke K, Bisgaard H. Cat exposure in early life
709　　　　　decreases asthma risk from the 17q21 high-risk variant. J Allergy Clin Immunol 2018;
710　　　　　141:1598-606.
711　12.　　Lee WM, Lemanske RF, Jr., Evans MD, Vang F, Pappas T, Gangnon R, et al. Human
712　　　　　rhinovirus species and season of infection determine illness severity. Am J Respir Crit
713　　　　　Care Med 2012; 186:886-91.
714　13.　　Nakagome K, Bochkov YA, Ashraf S, Brockman-Schneider RA, Evans MD, Pasic TR, et al.
715　　　　　Effects of rhinovirus species on viral replication and cytokine production. J Allergy Clin
716　　　　　Immunol 2014; 134:332-41.
717　14.　　Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al. A
718　　　　　genome-wide association study identifies CDHR3 as a susceptibility locus for early
719　　　　　childhood asthma with severe exacerbations. Nat Genet 2014; 46:51-5.

720 15. Bochkov YA, Watters K, Ashraf S, Griggs TF, Devries MK, Jackson DJ, et al. Cadherin-
721   related family member 3, a childhood asthma susceptibility gene product, mediates
722   rhinovirus C binding and replication. Proc Natl Acad Sci U S A 2015; 112:5485-90.
723 16. Bonnelykke K, Coleman AT, Evans MD, Thorsen J, Waage J, Vissing NH, et al. Cadherin-
724   related Family Member 3 Genetics and Rhinovirus C Respiratory Illnesses. Am J Respir
725   Crit Care Med 2018; 197:589-94.
726 17. Vrijheid M. The exposome: a new paradigm to study the impact of environment on
727   health. Thorax 2014; 69:876-8.
728 18. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus
729   bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J
730   Respir Crit Care Med 2000; 161:1501-7.
731 19. Welliver RC. RSV and chronic asthma. Lancet 1995; 346:789-90.
732 20. Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, et al. The severity-
733   dependent relationship of infant bronchiolitis on the risk and morbidity of early
734   childhood asthma. J Allergy Clin Immunol 2009; 123:1055-61.
735 21. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing
736   rhinovirus illnesses in early life predict asthma development in high-risk children. Am J
737   Respir Crit Care Med 2008; 178:667-72.
738 22. Lemanske RF, Jr., Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. Rhinovirus
739   illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol
740   2005; 116:571-7.
741 23. Lukkarinen M, Koistinen A, Turunen R, Lehtinen P, Vuorinen T, Jartti T. Rhinovirus-
742   induced first wheezing episode predicts atopic but not nonatopic asthma at school age. J
743   Allergy Clin Immunol 2017; 140:988-95.
744 24. Heymann PW, Carper HT, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, et al.
745   Viral infections in relation to age, atopy, and season of admission among children
746   hospitalized for wheezing. J Allergy Clin Immunol 2004; 114:239-47.
747 25. Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, et al. Rhinovirus
748   and respiratory syncytial virus in wheezing children requiring emergency care. IgE and
749   eosinophil analyses. Am J Respir Crit Care Med 1999; 159:785-90.
750 26. Soto-Quiros M, Avila L, Platts-Mills TA, Hunt JF, Erdman DD, Carper H, et al. High titers of
751   IgE antibody to dust mite allergen and risk for wheezing among asthmatic children
752   infected with rhinovirus. J Allergy Clin Immunol 2012; 129:1499-505 e5.
753 27. Beale J, Jayaraman A, Jackson DJ, Macintyre JDR, Edwards MR, Walton RP, et al.
754   Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic
755   pulmonary inflammation. Sci Transl Med 2014; 6:256ra134.
756 28. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, et al. Role of
757   deficient type III interferon-lambda production in asthma exacerbations. Nat Med 2006;
758   12:1023-6.
759 29. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-
760   33-Dependent Type 2 Inflammation during Rhinovirus-induced Asthma Exacerbations In
761   Vivo. Am J Respir Crit Care Med 2014; 190:1373-82.

762 30. Kennedy JL, Koziol-White CJ, Jeffus S, Rettiganti MR, Fisher P, Kurten M, et al. Effects of
763   rhinovirus 39 infection on airway hyperresponsiveness to carbachol in human airways
764   precision cut lung slices. J Allergy Clin Immunol 2018; 141:1887-90 e1.
765 31. Kennedy JL, Shaker M, McMeen V, Gern J, Carper H, Murphy D, et al. Comparison of
766   viral load in individuals with and without asthma during infections with rhinovirus. Am J
767   Respir Crit Care Med 2014; 189:532-9.
768 32. Khaitov MR, Laza-Stanca V, Edwards MR, Walton RP, Rohde G, Contoli M, et al.
769   Respiratory virus induction of alpha-, beta- and lambda-interferons in bronchial
770   epithelial cells and peripheral blood mononuclear cells. Allergy 2009; 64:375-86.
771 33. Laza-Stanca V, Message SD, Edwards MR, Parker HL, Zdrenghea MT, Kebadze T, et al.
772   The role of IL-15 deficiency in the pathogenesis of virus-induced asthma exacerbations.
773   PLoS Pathog 2011; 7:e1002114.
774 34. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, et al. Rhinovirus-
775   induced lower respiratory illness is increased in asthma and related to virus load and
776   Th1/2 cytokine and IL-10 production. Proc Natl Acad Sci U S A 2008; 105:13562-7.
777 35. Liu L, Pan Y, Zhu Y, Song Y, Su X, Yang L, et al. Association between rhinovirus wheezing
778   illness and the development of childhood asthma: a meta-analysis. BMJ Open 2017;
779   7:e013034.
780 36. Midulla F, Nicolai A, Ferrara M, Gentile F, Pierangeli A, Bonci E, et al. Recurrent
781   wheezing 36 months after bronchiolitis is associated with rhinovirus infections and
782   blood eosinophilia. Acta Paediatr 2014; 103:1094-9.
783 37. Rubner FJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, et al. Early life
784   rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence. J Allergy Clin
785   Immunol 2017; 139:501-7.
786 38. Ruotsalainen M, Hyvarinen MK, Piippo-Savolainen E, Korppi M. Adolescent asthma after
787   rhinovirus and respiratory syncytial virus bronchiolitis. Pediatr Pulmonol 2013; 48:633-9.
788 39. Schneider D, Hong JY, Popova AP, Bowman ER, Linn MJ, McLean AM, et al. Neonatal
789   rhinovirus infection induces mucous metaplasia and airways hyperresponsiveness. J
790   Immunol 2012; 188:2894-904.
791 40. Hong JY, Bentley JK, Chung Y, Lei J, Steenrod JM, Chen Q, et al. Neonatal rhinovirus
792   induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2
793   innate lymphoid cells. J Allergy Clin Immunol 2014; 134:429-39 e8.
794 41. Han M, Rajput C, Hong JY, Lei J, Hinde JL, Wu Q, et al. The Innate Cytokines IL-25, IL-33,
795   and TSLP Cooperate in the Induction of Type 2 Innate Lymphoid Cell Expansion and
796   Mucous Metaplasia in Rhinovirus-Infected Immature Mice. J Immunol 2017; 199:1308-
797   18.
798 42. Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Lee WM, et al. Evidence for a
799   causal relationship between allergic sensitization and rhinovirus wheezing in early life.
800   Am J Respir Crit Care Med 2012; 185:281-5.
801 43. Contoli M, Ito K, Padovani A, Poletti D, Marku B, Edwards MR, et al. Th2 cytokines impair
802   innate immune responses to rhinovirus in respiratory epithelial cells. Allergy 2015;
803   70:910-20.
804 44. Bochkov YA, Grindle K, Vang F, Evans MD, Gern JE. Improved molecular typing assay for
805   rhinovirus species A, B, and C. J Clin Microbiol 2014; 52:2461-71.

806 45. Miller EK, Hernandez JZ, Wimmenauer V, Shepherd BE, Hijano D, Libster R, et al. A
807   Mechanistic Role for Type III IFN-lambda1 in Asthma Exacerbations Mediated by Human
808   Rhinoviruses. Am J Respir Crit Care Med 2012; 185:508-16.
809 46. Altman MC, Reeves SR, Parker AR, Whalen E, Misura KM, Barrow KA, et al. Interferon
810   response to respiratory syncytial virus by bronchial epithelium from children with
811   asthma is inversely correlated with pulmonary function. J Allergy Clin Immunol 2017.
812 47. Raundhal M, Morse C, Khare A, Oriss TB, Milosevic J, Trudeau J, et al. High IFN-gamma
813   and low SLPI mark severe asthma in mice and humans. J Clin Invest 2015; 125:3037-50.
814 48. Custovic A, Belgrave D, Lin L, Bakhsoliani E, Telcian AG, Solari R, et al. Cytokine
815   Responses to Rhinovirus and Development of Asthma, Allergic Sensitization, and
816   Respiratory Infections during Childhood. Am J Respir Crit Care Med 2018; 197:1265-74.
817 49. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen
818   sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet
819   2006; 368:763-70.
820 50. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life
821   respiratory viral infections, atopic sensitization, and risk of subsequent development of
822   persistent asthma. J Allergy Clin Immunol 2007; 119:1105-10.
823 51. Gill MA, Bajwa G, George TA, Dong CC, Dougherty II, Jiang N, et al. Counterregulation
824   between the FcepsilonRI pathway and antiviral responses in human plasmacytoid
825   dendritic cells. J Immunol 2010; 184:5999-6006.
826 52. Esquivel A, Busse WW, Calatroni A, Togias AG, Grindle KG, Bochkov YA, et al. Effects of
827   Omalizumab on Rhinovirus Infections, Illnesses, and Exacerbations of Asthma. Am J
828   Respir Crit Care Med 2017; 196:985-92.
829 53. Inouye M, Teo SM, Tang H, Mok D, Judd L, Watts S, et al. Dynamics of airway microbiota
830   identify a critical window for interplay of pathogenic bacteria and allergic sensitization in
831   childhood respiratory disease. Cell Host & Microbe 2018.
832 54. Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, et al. Respiratory
833   syncytial virus in early life and risk of wheeze and allergy by age 13 years. The Lancet
834   1999; 354:541-5.
835 55. Henderson J, Hilliard TN, Sherriff A, Stalker D, Shammari NA, Thomas HM.
836   Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma,
837   atopy and wheeze: A longitudinal birth cohort study. Pediatric Allergy and Immunology
838   2005; 16:386-92.
839 56. Castro-Rodriguez JA, Forno E, Rodriguez-Martinez CE, Celedon JC. Risk and Protective
840   Factors for Childhood Asthma: What Is the Evidence? J Allergy Clin Immunol Pract 2016;
841   4:1111-22.
842 57. Rodriguez-Fernandez R, Tapia LI, Yang CF, Torres JP, Chavez-Bueno S, Garcia C, et al.
843   Respiratory Syncytial Virus Genotypes, Host Immune Profiles, and Disease Severity in
844   Young Children Hospitalized With Bronchiolitis. J Infect Dis 2017; 217:24-34.
845 58. de Steenhuijsen Piters WA, Heinonen S, Hasrat R, Bunsow E, Smith B, Suarez-Arrabal
846   MC, et al. Nasopharyngeal Microbiota, Host Transcriptome, and Disease Severity in
847   Children with Respiratory Syncytial Virus Infection. Am J Respir Crit Care Med 2016;
848   194:1104-15.

849   59.   Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T, et al. Evidence of a
850   causal role of winter virus infection during infancy in early childhood asthma. Am J
851   Respir Crit Care Med 2008; 178:1123-9.
852   60.   Thomsen SF, van der Sluis S, Stensballe LG, Posthuma D, Skytthe A, Kyvik KO, et al.
853   Exploring the association between severe respiratory syncytial virus infection and
854   asthma: a registry-based twin study. Am J Respir Crit Care Med 2009; 179:1091-7.
855   61.   Carroll KN, Gebretsadik T, Escobar GJ, Wu P, Li SX, Walsh EM, et al. Respiratory syncytial
856   virus immunoprophylaxis in high-risk infants and development of childhood asthma.
857   Journal of Allergy and Clinical Immunology 2017; 139:66-71.e3.
858   62.   Mochizuki H, Kusuda S, Okada K, Yoshihara S, Furuya H, Simoes EAF, et al. Palivizumab
859   Prophylaxis in Preterm Infants and Subsequent Recurrent Wheezing. Six-Year Follow-up
860   Study. Am J Respir Crit Care Med 2017; 196:29-38.
861   63.   Connors M, Giese NA, Kulkarni AB, Firestone CY, Morse HC, 3rd, Murphy BR. Enhanced
862   pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of
863   formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of
864   interleukin-4 (IL-4) and IL-10. J Virol 1994; 68:5321-5.
865   64.   Waris ME, Tsou C, Erdman DD, Zaki SR, Anderson LJ. Respiratory synctial virus infection
866   in BALB/c mice previously immunized with formalin-inactivated virus induces enhanced
867   pulmonary inflammatory response with a predominant Th2-like cytokine pattern. J Virol
868   1996; 70:2852-60.
869   65.   Johnson TR, Johnson JE, Roberts SR, Wertz GW, Parker RA, Graham BS. Priming with
870   secreted glycoprotein G of respiratory syncytial virus (RSV) augments interleukin-5
871   production and tissue eosinophilia after RSV challenge. J Virol 1998; 72:2871-80.
872   66.   Krishnamoorthy N, Khare A, Oriss TB, Raundhal M, Morse C, Yarlagadda M, et al. Early
873   infection with respiratory syncytial virus impairs regulatory T cell function and increases
874   susceptibility to allergic asthma. Nat Med 2012; 18:1525-30.
875   67.   Stier MT, Bloodworth MH, Toki S, Newcomb DC, Goleniewska K, Boyd KL, et al.
876   Respiratory syncytial virus infection activates IL-13-producing group 2 innate lymphoid
877   cells through thymic stromal lymphopoietin. J Allergy Clin Immunol 2016; 138:814-24
878   e11.
879   68.   Siegle JS, Hansbro N, Herbert C, Rosenberg HF, Domachowske JB, Asquith KL, et al. Early-
880   life viral infection and allergen exposure interact to induce an asthmatic phenotype in
881   mice. Respir Res 2010; 11:14.
882   69.   Gill MA, Liu AH, Calatroni A, Krouse RZ, Shao B, Schiltz A, et al. Enhanced plasmacytoid
883   dendritic cell antiviral responses after omalizumab. J Allergy Clin Immunol 2018;
884   141:1735-43 e9.
885   70.   Olenec JP, Kim WK, Lee WM, Vang F, Pappas TE, Salazar LE, et al. Weekly monitoring of
886   children with asthma for infections and illness during common cold seasons. J Allergy
887   Clin Immunol 2010; 125:1001-6.
888   71.   Kloepfer KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD, et al. Detection of
889   pathogenic bacteria during rhinovirus infection is associated with increased respiratory
890   symptoms and asthma exacerbations. J Allergy Clin Immunol 2014; 133:1301-7, 7 e1-3.

891   72.   Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N, et al. The infant nasopharyngeal
892   microbiome impacts severity of lower respiratory infection and risk of asthma
893   development. Cell Host Microbe 2015; 17:704-15.
894   73.   Hyde ER, Petrosino JF, Piedra PA, Camargo CA, Jr., Espinola JA, Mansbach JM.
895   Nasopharyngeal Proteobacteria are associated with viral etiology and acute wheezing in
896   children with severe bronchiolitis. J Allergy Clin Immunol 2013.
897   74.   Bosch A, de Steenhuijsen Piters WAA, van Houten MA, Chu M, Biesbroek G, Kool J, et al.
898   Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health
899   Consequences. A Prospective Cohort Study. Am J Respir Crit Care Med 2017; 196:1582-
900   90.
901   75.   Kloepfer KM, Sarsani VK, Poroyko V, Lee WM, Pappas TE, Kang T, et al. Community-
902   acquired rhinovirus infection is associated with changes in the airway microbiome. J
903   Allergy Clin Immunol 2017; 140:312-5 e8.
904   76.   Hasegawa K, Mansbach JM, Ajami NJ, Espinola JA, Henke DM, Petrosino JF, et al.
905   Association of nasopharyngeal microbiota profiles with bronchiolitis severity in infants
906   hospitalised for bronchiolitis. Eur Respir J 2016; 48:1329-39.
907   77.   Rosas-Salazar C, Shilts MH, Tovchigrechko A, Schobel S, Chappell JD, Larkin EK, et al.
908   Nasopharyngeal Lactobacillus is associated with a reduced risk of childhood wheezing
909   illnesses following acute respiratory syncytial virus infection in infancy. J Allergy Clin
910   Immunol 2018.
911   78.   Rosas-Salazar C, Shilts MH, Tovchigrechko A, Schobel S, Chappell JD, Larkin EK, et al.
912   Differences in the Nasopharyngeal Microbiome During Acute Respiratory Tract Infection
913   With Human Rhinovirus and Respiratory Syncytial Virus in Infancy. J Infect Dis 2016;
914   214:1924-8.
915   79.   Folsgaard NV, Schjorring S, Chawes BL, Rasmussen MA, Krogfelt KA, Brix S, et al.
916   Pathogenic bacteria colonizing the airways in asymptomatic neonates stimulates topical
917   inflammatory mediator release. Am J Respir Crit Care Med 2013; 187:589-95.
918   80.   Tomosada Y, Chiba E, Zelaya H, Takahashi T, Tsukida K, Kitazawa H, et al. Nasally
919   administered Lactobacillus rhamnosus strains differentially modulate respiratory
920   antiviral immune responses and induce protection against respiratory syncytial virus
921   infection. BMC Immunol 2013; 14:40.
922   81.   Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of
923   Bacterial to Host Cells in Humans. Cell 2016; 164:337-40.
924   82.   Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, et al.
925   Cospeciation of gut microbiota with hominids. Science 2016; 353:380-2.
926   83.   Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation
927   in the human colon. J Appl Bacteriol 1991; 70:443-59.
928   84.   Laforest-Lapointe I, Arrieta MC. Patterns of Early-Life Gut Microbial Colonization during
929   Human Immune Development: An Ecological Perspective. Front Immunol 2017; 8:788.
930   85.   Fujimura KE, Lynch SV. Microbiota in allergy and asthma and the emerging relationship
931   with the gut microbiome. Cell Host Microbe 2015; 17:592-602.
932   86.   Sokolowska M, Frei R, Lunjani N, Akdis CA, O'Mahony L. Microbiome and asthma.
933   Asthma Res Pract 2018; 4:1.

934 87. McAleer JP, Kolls JK. Contributions of the intestinal microbiome in lung immunity. Eur J
935 Immunol 2018; 48:39-49.
936 88. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, et al. Cytokine response
937 patterns, exposure to viruses, and respiratory infections in the first year of life.
938 Am.J.Respir.Crit Care Med. 2004; 170:175-80.
939 89. Sumino K, Tucker J, Shahab M, Jaffee KF, Visness CM, Gern JE, et al. Antiviral IFN-gamma
940 responses of monocytes at birth predict respiratory tract illness in the first year of life.
941 J.Allergy Clin.Immunol. 2012; 129:1267-73.
942 90. Gern JE, Brooks GD, Meyer P, Chang A, Shen K, Evans MD, et al. Bidirectional
943 interactions between viral respiratory illnesses and cytokine responses in the first year
944 of life. J Allergy Clin Immunol 2006; 117:72-8.
945 91. Ly NP, Rifas-Shiman SL, Litonjua AA, Tzianabos AO, Schaub B, Ruiz-Perez B, et al. Cord
946 blood cytokines and acute lower respiratory illnesses in the first year of life. Pediatrics
947 2007; 119:e171-8.
948 92. Macaubas C, de Klerk NH, Holt BJ, Wee C, Kendall G, Firth M, et al. Association between
949 antenatal cytokine production and the development of atopy and asthma at age 6 years.
950 Lancet 2003; 362:1192-7.
951 93. Pelgrom LR, Everts B. Metabolic control of type 2 immunity. Eur J Immunol 2017;
952 47:1266-75.
953 94. Donovan BM, Ryckman KK, Breheny PJ, Gebretsadik T, Turi KN, Larkin EK, et al.
954 Association of newborn screening metabolites with risk of wheezing in childhood.
955 Pediatr Res 2018.
956 95. Bufford JD, Reardon CL, Li Z, Roberg KA, Dasilva D, Eggleston PA, et al. Effects of dog
957 ownership in early childhood on immune development and atopic diseases. Clin Exp
958 Allergy 2008; 38:1635-43.
959 96. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life
960 and risk of allergic sensitization at 6 to 7 years of age. JAMA 2002; 288:963-72.
961 97. Ludka-Gaulke T, Ghera P, Waring SC, Keifer M, Seroogy C, Gern JE, et al. Farm exposure
962 in early childhood is associated with a lower risk of severe respiratory illnesses. J Allergy
963 Clin Immunol 2018; 141:454-6 e4.
964 98. Lynch SV, Wood RA, Boushey H, Bacharier LB, Bloomberg GR, Kattan M, et al. Effects of
965 early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban
966 children. J Allergy Clin Immunol 2014; 134:593-601 e12.
967 99. Gern JE, Calatroni A, Jaffee KF, Lynn H, Dresen A, Cruikshank WW, et al. Patterns of
968 immune development in urban preschoolers with recurrent wheeze and/or atopy. J
969 Allergy Clin Immunol 2017.
970 100. Schaub B, Liu J, Hoppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure
971 modulates neonatal immune mechanisms through regulatory T cells. J Allergy Clin
972 Immunol 2009; 123:774-82 e5.
973 101. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal
974 bacteria protect against food allergen sensitization. Proc Natl Acad Sci U S A 2014;
975 111:13145-50.

976 102. Manfredo Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, et al.
977   Translocation of a gut pathobiont drives autoimmunity in mice and humans. Science
978   2018; 359:1156-61.
979 103. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota
980   regulates immune defense against respiratory tract influenza A virus infection. Proc Natl
981   Acad Sci U S A 2011; 108:5354-9.
982 104. Fujimura KE, Demoor T, Rauch M, Faruqi AA, Jang S, Johnson CC, et al. House dust
983   exposure mediates gut microbiome Lactobacillus enrichment and airway immune
984   defense against allergens and virus infection. Proc Natl Acad Sci U S A 2014; 111:805-10.
985 105. Ciaccio CE. Modulating the microbiome: The future of allergy therapeutics? Ann Allergy
986   Asthma Immunol 2019; 122:233-5.
987 106. Wang HT, Anvari S, Anagnostou K. The Role of Probiotics in Preventing Allergic Disease.
988   Children (Basel) 2019; 6.
989 107. Chiu CY, Chan YL, Tsai MH, Wang CJ, Chiang MH, Chiu CC. Gut microbial dysbiosis is
990   associated with allergen-specific IgE responses in young children with airway allergies.
991   World Allergy Organ J 2019; 12:100021.
992 108. Beigelman A, Bacharier LB. Early-life respiratory infections and asthma development:
993   role in disease pathogenesis and potential targets for disease prevention. Curr Opin
994   Allergy Clin Immunol 2016; 16:172-8.
995 109. Blanken MO, Rovers MM, Molenaar JM, Winkler-Seinstra PL, Meijer A, Kimpen JL, et al.
996   Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. N Engl J
997   Med 2013; 368:1791-9.
998 110. Scheltema NM, Nibbelke EE, Pouw J, Blanken MO, Rovers MM, Naaktgeboren CA, et al.
999   Respiratory syncytial virus prevention and asthma in healthy preterm infants: a
1000   randomised controlled trial. Lancet Respir Med 2018; 6:257-64.
1001 111. Beigelman A, Isaacson-Schmid M, Sajol G, Baty J, Rodriguez OM, Leege E, et al.
1002   Randomized trial to evaluate azithromycin's effects on serum and upper airway IL-8
1003   levels and recurrent wheezing in infants with respiratory syncytial virus bronchiolitis. J
1004   Allergy Clin Immunol 2015; 135:1171-8 e1.
1005 112. Zhou Y, Bacharier LB, Isaacson-Schmid M, Baty J, Schechtman KB, Sajol G, et al.
1006   Azithromycin therapy during respiratory syncytial virus bronchiolitis: Upper airway
1007   microbiome alterations and subsequent recurrent wheeze. J Allergy Clin Immunol 2016;
1008   138:1215-9 e5.
1009 113. Chawes BL, Bonnelykke K, Stokholm J, Vissing NH, Bjarnadottir E, Schoos AM, et al.
1010   Effect of Vitamin D3 Supplementation During Pregnancy on Risk of Persistent Wheeze in
1011   the Offspring: A Randomized Clinical Trial. JAMA 2016; 315:353-61.
1012 114. Litonjua AA, Carey VJ, Laranjo N, Harshfield BJ, McElrath TF, O'Connor GT, et al. Effect of
1013   Prenatal Supplementation With Vitamin D on Asthma or Recurrent Wheezing in
1014   Offspring by Age 3 Years: The VDAART Randomized Clinical Trial. JAMA 2016; 315:362-
1015   70.
1016 115. Wolsk HM, Chawes BL, Litonjua AA, Hollis BW, Waage J, Stokholm J, et al. Prenatal
1017   vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood:
1018   A combined analysis of two randomized controlled trials. PLoS One 2017; 12:e0186657.

1019   116.   Mirzakhani H, Al-Garawi A, Weiss ST, Litonjua AA. Vitamin D and the development of
1020   allergic disease: how important is it? Clinical and Experimental Allergy 2015; 45:114-25.
1021   117.   Bisgaard H, Stokholm J, Chawes BL, Vissing NH, Bjarnadottir E, Schoos AM, et al. Fish Oil-
1022   Derived Fatty Acids in Pregnancy and Wheeze and Asthma in Offspring. N Engl J Med
1023   2016; 375:2530-9.
1024   118.   Del-Rio-Navarro BE, Espinosa Rosales F, Flenady V, Sienra-Monge JJ. Immunostimulants
1025   for preventing respiratory tract infection in children. Cochrane Database Syst Rev
1026   2006:CD004974.
1027   119.   Kew KM, Quinn M, Quon BS, Ducharme FM. Increased versus stable doses of inhaled
1028   corticosteroids for exacerbations of chronic asthma in adults and children. Cochrane
1029   Database Syst Rev 2016:CD007524.
1030   120.   Jackson DJ, Bacharier LB, Mauger DT, Boehmer S, Beigelman A, Chmiel JF, et al.
1031   Quintupling Inhaled Glucocorticoids to Prevent Childhood Asthma Exacerbations. N Engl
1032   J Med 2018; 378:891-901.
1033   121.   Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum
1034   vitamin D levels and markers of severity of childhood asthma in costa rica. Am J Respir
1035   Crit Care Med 2009; 179:765-71.
1036   122.   Denlinger LC, King TS, Cardet JC, Craig T, Holguin F, Jackson DJ, et al. Vitamin D
1037   Supplementation and the Risk of Colds in Patients with Asthma. Am J Respir Crit Care
1038   Med 2016; 193:634-41.
1039   123.   Castro M, King TS, Kunselman SJ, Cabana MD, Denlinger L, Holguin F, et al. Effect of
1040   vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower
1041   vitamin D levels: the VIDA randomized clinical trial. JAMA 2014; 311:2083-91.
1042   124.   Jolliffe DA, Greenberg L, Hooper RL, Griffiths CJ, Camargo CA, Jr., Kerley CP, et al.
1043   Vitamin D supplementation to prevent asthma exacerbations: a systematic review and
1044   meta-analysis of individual participant data. Lancet Respir Med 2017; 5:881-90.
1045   125.   Bacharier LB, Guilbert TW, Mauger DT, Boehmer S, Beigelman A, Fitzpatrick AM, et al.
1046   Early Administration of Azithromycin and Prevention of Severe Lower Respiratory Tract
1047   Illnesses in Preschool Children With a History of Such Illnesses: A Randomized Clinical
1048   Trial. JAMA 2015; 314:2034-44.
1049   126.   Stokholm J, Chawes BL, Vissing NH, Bjarnadottir E, Pedersen TM, Vinding RK, et al.
1050   Azithromycin for episodes with asthma-like symptoms in young children aged 1-3 years:
1051   a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 2016; 4:19-26.
1052   127.   Kantor DB, Stenquist N, McDonald MC, Schultz BJ, Hauptman M, Smallwood CD, et al.
1053   Rhinovirus and serum IgE are associated with acute asthma exacerbation severity in
1054   children. J Allergy Clin Immunol 2016; 138:1467-71 e9.
1055   128.   Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ, Jr., Calatroni A, et al. Preseasonal
1056   treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall
1057   asthma exacerbations. J Allergy Clin Immunol 2015; 136:1476-85.
1058   129.   Kantor DB, McDonald MC, Stenquist N, Schultz BJ, Smallwood CD, Nelson KA, et al.
1059   Omalizumab Is Associated with Reduced Acute Severity of Rhinovirus-triggered Asthma
1060   Exacerbation. Am J Respir Crit Care Med 2016; 194:1552-5.
1061   130.   Welliver RC, Kaul TN, Ogra PL. The appearance of cell-bound IgE in respiratory-tract
1062   epithelium after respiratory-syncytial-virus infection. N Engl J Med 1980; 303:1198-202.

1063   131.   Welliver RC, Wong DT, Sun M, Middleton E, Jr., Vaughan RS, Ogra PL. The development
1064   of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal
1065   secretions after infection. N Engl J Med 1981; 305:841-6.
1066   132.   Tam JS, Jackson WT, Hunter D, Proud D, Grayson MH. Rhinovirus specific IgE can be
1067   detected in human sera. J Allergy Clin Immunol 2013; 132:1241-3.
1068
1069

1070
1071 Table 1. Review of most salient points
1072
1073 1. Polymorphisms in several antiviral and innate immune genes have been linked to
1074 susceptibility to respiratory viruses, infection severity, and virus-induced asthma exacerbations,
1075 and have been replicated across multiple cohorts; these genes include *STAT4, JAK2, MX1,*
1076 *VDR, DDX58, and EIF2AK2.*
1077
1078 2. Rhinovirus virulence varies by species; RV-A and RV-C are more likely to cause illnesses,
1079 wheezing and lower respiratory tract infection compared to RV-B.
1080
1081 3. RV infection leads to expression of epithelial derived cytokines IL-25, IL-33, and TSLP and
1082 an increase in ILC2s as an important source of airway IL-13; blocking these pathways with anti-
1083 IL-25 attenuates neonatal RV-induced AHR and mucous cell metaplasia in mice.
1084
1085 4. Two prevention studies using palivizumab (a monoclonal antibody directed against RSV) in
1086 high-risk infants found that prevention of more severe RSV-illnesses decreased the risk of
1087 childhood recurrent wheezing but not asthma development.
1088
1089 5. In several infant cross-sectional and cohort studies, the presence of *Streptococcus,*
1090 *Moraxella*, or *Haemophilus* during upper respiratory infection increases the likelihood that the
1091 infant will have lower airway symptoms; studies examining airway bacteria during RSV
1092 bronchiolitis have reported links between an increased abundance of *Streptococcus* and
1093 *Haemophilus*, while in contrast, RV-bronchiolitis is associated with an increased abundance of
1094 *Moraxella* and *Haemophilus.*
1095
1096 6. The presence of *H. influenzae* in the infant airway prior to viral infection is associated with
1097 increased expression of local inflammatory cytokines suggesting that a link exists between
1098 bacteria and airway inflammation; in contrast, mice receiving intranasal administration of
1099 *Lactobacillus rhamnosus* prior to viral infection have an enhanced antiviral immune response[80],
1100 suggesting that some bacteria may protect the airway and help prevent viral infection.
1101
1102 7. The gut microbiome also regulate pulmonary anti-viral immunity; in a murine model intact
1103 commensal bacteria in the gut were required for adaptive immune responses to respiratory
1104 influenza virus infection.
1105
1106 8. Unique components of the viral genome contribute to respiratory illness and knowledge of
1107 these factors may also assist in vaccine and therapeutic strategies aimed at the proteins
1108 responsible for specific disease characteristics.
1109
1110 9. Omalizumab, a humanized monoclonal antibody that selectively binds to IgE, decreased fall
1111 asthma exacerbations in atopic asthmatics and increased IFN-α responses to RV *ex vivo* in the
1112 Preventative Omalizumab or Step-Up Therapy for Fall Exacerbations (PROSE) study, whereas
1113 boosting ICS did not reduce risk.
1114
1115 10. A recent meta-analysis that combined two clinical trials (VDAART, COPSAC2010)
1116 investigated whether maternal vitamin D supplementation (2400 IU/day, 4000IU/day) during
1117 pregnancy revealed that this intervention resulted in a 25% significant reduction in
1118 asthma/recurrent wheeze risk during the first 3 years of life.
1119

1120   Table 3. Future/ongoing interventional studies examining treatments for viral triggered
1121   asthma
1122

Study Title	Study Population	Intervention	Primary Outcome Measurement	Estimated Completion Date
Vitamin D In the Prevention of Viral-Induced Asthma in preschoolers	children age 1-<6 with recurrent cold triggered asthma attacks, expected enrollment 865 subjects	Baseline and 3.5 month high dose vitamin D 100,000 IU and daily Vitamin D dose 400 IU OR placebo	Number of courses of rescue oral steroids (OCS) over 7 months	December 2022 *enrolling*
Azithromycin to Prevent Wheezing Following Severe RSV Bronchiolitis II	children 1-18 months of age, hospitalized due to RSV bronchiolitis, expected enrollment 200 subjects	Azithromycin (10 mg/kg x 7 days followed by 5 mg/kg x 7days) OR placebo	Time to occurrence of a 3rd episode of post-RSV wheezing, observation over 48 months	December 2021 *not yet enrolling*

1123 Figure 1. Immune responses to virus in the allergic asthmatic host. In the healthy host, anti-viral
1124 IFN responses control and clear respiratory viral infections. In allergic asthmatics, the release of
1125 the type 2-skewing cytokines TSLP, IL-25, and IL-33 promote the induction of Th2 cytokines
1126 and the suppression of IFN responses, in addition to promoting airway hyperreactivity (AHR)
1127 and increased mucus and IgE production. Furthermore, IgE has the capacity to suppress IFN-α
1128 production by Plasmacytoid DCs (pDCs).
1129
1130
1131

Table 1. Review of most salient points

1. Polymorphisms in several antiviral and innate immune genes have been linked to susceptibility to respiratory viruses, infection severity, and virus-induced asthma exacerbations, and have been replicated across multiple cohorts; these genes include *STAT4, JAK2, MX1, VDR, DDX58, and EIF2AK2.*

2. Rhinovirus virulence varies by species; RV-A and RV-C are more likely to cause illnesses, wheezing and lower respiratory tract infection compared to RV-B.

3. RV infection leads to expression of epithelial derived cytokines IL-25, IL-33, and TSLP and an increase in ILC2s as an important source of airway IL-13; blocking these pathways with anti-IL-25 attenuates neonatal RV-induced AHR and mucous cell metaplasia in mice.

4. Two prevention studies using palivizumab (a monoclonal antibody directed against RSV) in high-risk infants found that prevention of more severe RSV-illnesses decreased the risk of childhood recurrent wheezing but not asthma development.

5. In several infant cross-sectional and cohort studies, the presence of *Streptococcus*, *Moraxella*, or *Haemophilus* during upper respiratory infection increases the likelihood that the infant will have lower airway symptoms; studies examining airway bacteria during RSV bronchiolitis have reported links between an increased abundance of *Streptococcus* and *Haemophilus*, while in contrast, RV-bronchiolitis is associated with an increased abundance of *Moraxella* and *Haemophilus.*

6. The presence of *H. influenzae* in the infant airway prior to viral infection is associated with increased expression of local inflammatory cytokines suggesting that a link exists between bacteria and airway inflammation; in contrast, mice receiving intranasal administration of *Lactobacillus rhamnosus* prior to viral infection have an enhanced antiviral immune response[80], suggesting that some bacteria may protect the airway and help prevent viral infection.

7. The gut microbiome also regulate pulmonary anti-viral immunity; in a murine model intact commensal bacteria in the gut were required for adaptive immune responses to respiratory influenza virus infection.

8. Unique components of the viral genome contribute to respiratory illness and knowledge of these factors may also assist in vaccine and therapeutic strategies aimed at the proteins responsible for specific disease characteristics.

9. Omalizumab, a humanized monoclonal antibody that selectively binds to IgE, decreased fall asthma exacerbations in atopic asthmatics and increased IFN-α responses to RV *ex vivo* in the Preventative Omalizumab or Step-Up Therapy for Fall Exacerbations (PROSE) study, whereas boosting ICS did not reduce risk.

10. A recent meta-analysis that combined two clinical trials (VDAART, COPSAC2010) investigated whether maternal vitamin D supplementation (2400 IU/day, 4000IU/day) during pregnancy revealed that this intervention resulted in a 25% significant reduction in asthma/recurrent wheeze risk during the first 3 years of life.

Table 2. Polymorphisms in several antiviral and innate immune genes have been linked to susceptibility to respiratory viruses, infection severity, and virus-induced asthma exacerbations, and have been replicated across multiple cohorts.

Gene	Function
STAT4	Transcription factor required for IL-12 signaling in the development of Th1 cells from naïve CD4 T cells
JAK2	A non-receptor tyrosine kinase critical for signaling of the GM-CSF, gp130, and single chain receptor families.
MX1	Responsible for the antiviral state against influenza infection
VDR	Vitamin D receptor
DDX58	Involved in antiviral signaling in response to viruses containing a dsDNA genome
EIF2AK2	Innate antiviral immune response to viral infection that can trigger apoptosis via FADD-mediated caspase 8
IRF7	Critical role in the innate immune response against DNA and RNA viruses
IFIH1	Provides instructions for making the MDA5 protein that has a critical role in innate antiviral immunity
IFNA5	One of the type I IFN-α isoforms that has antiviral activities
NOS2	Nitric oxide synthase gene that mediates the antiviral activity of IFN-γ
ADAM33	Member of the ADAM (a disintegrin and metalloprotease domain) family identified as a major susceptibility gene in asthma
IL4R	Interleukin 4 receptor through which IL-4 and IL-13 signal to induce IgE class switching and airway mucus metaplasia
CD14	Multiple functions, one of which is critical for TLR signaling in host defense
TNF	Tumor necrosis factor that is produced in abundance by mast cells and has roles in cell survival and proliferation
IL13	Important in airway mucous cell metaplasia, airways responsiveness, VCAM expression
IL1RL1	One subunit of the receptor for IL-33, which can activate ILC2 and promote CD4 T cell differentiation toward Th2 phenotype
CDHR3	Cadherin that is the receptor for rhinovirus

Table 3. Future/ongoing interventional studies examining treatments for viral triggered asthma

Study Title	Study Population	Intervention	Primary Outcome Measurement	Estimated Completion Date
Vitamin D In the Prevention of Viral-Induced Asthma in preschoolers	children age 1-<6 with recurrent cold triggered asthma attacks, expected enrollment 865 subjects	Baseline and 3.5 month high dose vitamin D 100,000 IU and daily Vitamin D dose 400 IU OR placebo	Number of courses of rescue oral steroids (OCS) over 7 months	December 2022 *enrolling*
Azithromycin to Prevent Wheezing Following Severe RSV Bronchiolitis II	children 1-18 months of age, hospitalized due to RSV bronchiolitis, expected enrollment 200 subjects	Azithromycin (10 mg/kg x 7 days followed by 5 mg/kg x 7days) OR placebo	Time to occurrence of a 3rd episode of post-RSV wheezing, observation over 48 months	December 2021 *not yet enrolling*

HEALTHY

ALLERGIC ASTHMA

IFN-γ

Th1

Virus

Allergen

Goblet
Cell

AHR

TSLP
IL-25
IL-33

Type I & III
IFNs

Th2

ILC2

pDC

IFN-α

IL-4 + IL-13

IgE

B