

Current Opinion in Allergy & Clinical Immunology

Host-Microbiome Intestinal Interactions during Early Life: Considerations for Atopy and Asthma Development

--Manuscript Draft--

Manuscript Number:	ACI200212R2
Full Title:	Host-Microbiome Intestinal Interactions during Early Life: Considerations for Atopy and Asthma Development
Article Type:	Review Article
Corresponding Author:	Marie-Claire Arrieta University of Calgary Cumming School of Medicine Calgary, CANADA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Calgary Cumming School of Medicine
Corresponding Author's Secondary Institution:	
First Author:	Veronika Kuchařová Pettersen, Ph.D.
First Author Secondary Information:	
Order of Authors:	Veronika Kuchařová Pettersen, Ph.D. Marie-Claire Arrieta, Ph.D.
Order of Authors Secondary Information:	

1 **Host-Microbiome Intestinal Interactions during Early Life: Considerations for**
2 **Atopy and Asthma Development**

3 **Veronika Kuchařová Pettersen^{1,2,3,4} and Marie-Claire Arrieta^{1,2,3*}**

4 ¹ Department of Physiology and Pharmacology, University of Calgary, Calgary,
5 Alberta, Canada.

6 ² Department of Pediatrics, University of Calgary, Calgary, Alberta, Canada.

7 ³ International Microbiome Centre, Cumming School of Medicine, Health Sciences
8 Centre University of Calgary, Calgary, Alberta, Canada.

9 ⁴ Department of Clinical Medicine, UiT - The Arctic University of Norway,
10 Tromsø, Norway

11 *Corresponding author

12 Name: Marie-Claire Arrieta

13 Address: University of Calgary, Health Research Innovation Centre, 3330

14 Hospital Drive N.W., Calgary T2N 4N1, Alberta, Canada

15 Telephone number: +1 403 220-4566

16 Email address: marie.arrieta@ucalgary.ca

17 *Keywords:* Early Life Gut Microbiome, Atopic Asthma, Treg/Th17/Th2 Balance,
18 Innate Lymphoid Cells, HPA Axis

19 Manuscript word count (excluding abstract, references, key points, and figure
20 legends): 3410

21

22 **Abstract**

23 **Purpose of review:** The body's largest microbial community, the gut
24 microbiome, is in contact with mucosal surfaces populated with epithelial,
25 immune, endocrine and nerve cells, all of which sense and respond to
26 microbial signals. These mutual interactions have led to a functional co-
27 evolution between the microbes and human physiology. Examples of co-
28 adaptation are anaerobes *Bifidobacteria* and *Bacteroides*, which have adjusted
29 their metabolism to dietary components of human milk, and infant immune
30 development, which has evolved to become reliant on the presence of
31 beneficial microbes. Current research suggests that specific composition of
32 the early-life gut microbiome aligns with the maturation of host immunity.
33 Disruptions of natural microbial succession patterns during gut colonization
34 are a consistent feature of immune-mediated diseases, including atopy and
35 asthma.

36 **Recent findings:** Here we catalog recent birth cohorts documenting
37 associations between immune dysregulation and microbial alterations, and
38 summarize the evidence supporting the role of the gut microbiome as an
39 etiological determinant of immune-mediated allergic diseases.

40 **Summary:** Ecological concepts that describe microbial dynamics in the context
41 of the host environment, and a portray of immune and neuroendocrine signaling
42 induced by host-microbiome interactions, have become indispensable in
43 describing the molecular role of early-life microbiome in atopy and asthma
44 susceptibility.

45

46 **Introduction**

47 The human gastrointestinal tract hosts the most abundant and diverse
48 community of microorganisms in the body, the gut microbiome (1). Many of
49 these microbial species interact with the intestinal mucosa that includes the
50 gut-associated lymphoid tissue (GALT), composed of more than 70% of all host
51 immune cells. Besides local interactions, microbes modulate cells in more
52 distant tissues and organs through their metabolites and other bioactive
53 molecules that enter the bloodstream. Pioneering studies with germ-free (GF)
54 animals were first to show that the absence of commensal microbes profoundly
55 alters the immune system's structural and functional development (2, 3).
56 Besides defects in lymphoid tissue within the spleen, thymus, and lymph
57 nodes, the GALT of GF animals display structural abnormalities near the
58 mucosal interface (4, 5) and an immune phenotype with a distorted ratio of
59 different T cell types (6). These deficits can be fully corrected by
60 introducing commensal microbiota exclusively during early life (7, 8), firmly
61 establishing that postnatal microbial colonization modulates the immune
62 system development.

63 An increasing number of studies is drawing attention to the microbiome as an
64 essential element determining the transition from health to disease and *vice*
65 *versa* (9). Epidemiological research on the effects of prenatal and postnatal
66 exposures has pointed out the association between perturbations of the gut
67 microbiome composition early in life and immunological dysregulation
68 affecting the risk of allergic diseases such as atopy and asthma (10-13).
69 Infants at increased risk of childhood atopy and/or asthma have
70 characteristic gut microbiome that exhibits depletion of specific bacterial
71 genera, fungal expansion and altered microbial metabolic function (**Table 1**).
72 In this review, we outline the current ecological understanding of early-life
73 interactions between the host and the gut microbiome that modulate immune

74 responses relevant to the development of atopy and asthma. We discuss how
75 microbiota sets the tone of allergen-specific responses as an immunological
76 priming event, as well as the roles of specific type 2 T helper cells (Th2)
77 and innate lymphoid cells. Lastly, we review recently revealed microbiome-
78 derived signals that impact the neuroendocrine system, which is capable of
79 modulating immune mechanisms in allergic responses, further underscoring the
80 overall complexity of allergic diseases etiology (**Figure 1**).

81 **Gut microbiome maturation and adaptation during early life**

82 The human host and its microbiome have coevolved in a complex relationship
83 that combines the host control of the microbial growth and microbial
84 competition for resources in the host environment (14, 15). This process has
85 led to a mutualistic symbiosis in which the microbiome augments host
86 physiological processes, and the host provides a nutritious and hospitable
87 environment for the microbes. The gut microbiome develops with age and
88 reflects the history of exposures to external factors, beginning with those
89 encountered during pregnancy (16). In the case of vaginal birth, the infant
90 microbiota composition is initially driven by selective seeding with maternal
91 gut strains (17, 18) and becomes gradually dominated by anaerobic species of
92 the *Bifidobacteria* and *Bacteroides* genera. The maturation of the gut
93 microbiome appears to happen in an orchestrated manner, and the timing of
94 microbial succession may be biologically determined (19, 20). Integrative
95 analyses of metagenomic data from 34 longitudinal studies worldwide revealed
96 common patterns in the relative abundance of the five most abundant bacterial
97 taxa in vaginally born infants. The same bacteria displayed delayed
98 colonization in infants born by caesarian section (C-section), as reported
99 previously (21). By the age of 12 months, major differences in the gut
100 microbiota composition caused by the mode of birth seem to disappear, and
101 this is also true for microbiomes affected by early-life antibiotic use (19).

102 Considering that the factors that drastically alter the gut microbiota
103 composition, including C-section, formula feeding, and antibiotic use, are
104 also well-established risk factors for asthma (22), it is likely that even
105 transient differences in the microbiota succession pattern may have long-term
106 effects on the immunological development of the host.

107 Applying the theoretical framework of microbial and community ecology can
108 help explain the connection between early life microbiome composition and
109 later health outcomes. An experimental study that compared sequential order
110 of microbial colonization in mice showed that the timing of bacterial arrival
111 in the gut has lasting effects on the overall composition of the microbiota
112 (23). This phenomenon, also known as priority effects, influenced how the
113 bacterial community assembled and how ecologically successful the individual
114 colonizers were. Human longitudinal studies provide additional evidence that
115 discernible early life microbiomes associate with different microbial
116 successional trajectories and health outcomes (**Table 1**). For example, infants
117 at high risk of asthma differ from low-risk babies by a distinct meconium
118 microbiota and a delay in the gut microbiota diversification over the first
119 year of life (13). Pioneer microbial species that initially populate the
120 infant gut might, therefore, not only impact the ecological succession of
121 microbes, and the resulting microbiome functional traits but very likely also
122 have a strong influence on immune tolerance and inflammation (1, 24, 25).

123 Another characteristic of infant gut microbiome is its low resilience, *i.e.*,
124 a reduced capacity of the microbial ecosystem to maintain and return to a
125 steady state in response to an external perturbation (1). The gut microbiome
126 during early life displays a lower species richness and overall microbial
127 diversity in contrast to the adult gut microbiome (26), in which a large
128 number of bacterial strains perform similar functions (27). Compared to the
129 substantial functional redundancy observed in adults, infant microbial

130 communities do not have the same functional overlap and are more prone to
131 loss of composition and functional traits upon external disturbances. This
132 aspect makes the infant gut microbiome highly unstable during the first year
133 of life.

134 One of the first colonizers of the human intestine that commonly dominate the
135 gut during breastfeeding and dissipate through life are *Bifidobacteria*.
136 Normal immune maturation appears to be dependent on this bacterial genus,
137 since atopic infants display reduced bifidobacterial levels in their stool
138 (28), and airway inflammation in murine model of asthma can be reduced by gut
139 colonization with a *B. breve* strain (29). From an evolutionary perspective,
140 increased abundance of maternal gut bifidobacteria during pregnancy
141 facilitates their vertical transmission from mother to newborn (30). The
142 species colonization success is further enhanced by their unique ability to
143 metabolize human milk oligosaccharides (31). A current study by Duranti *et*
144 *al.* looked into genetic adaptations that promote bifidobacteria-dominant
145 microbiome during infancy, and illustrated how different bifidobacterial taxa
146 have co-evolved to maximize their colonization capabilities through efficient
147 resource sharing (32).

148 **Adaptation of immune system to intestinal microbes in the context of atopic**
149 **asthma etiology**

150 Vaginal delivery and subsequent breastfeeding period reinforces
151 *Bifidobacterium* as a keystone species of the infant microbiome (33). High
152 bifidobacterial levels, which can reach up to 80% of the total gut microbiota
153 (34), temporally correlate with critical stages of immune cell maturation
154 (35, 36). Along with other prominent human commensals such as *Bacteroides*
155 *fragilis* (37), *Lactobacillus reuteri* (38), and *Clostridium* spp. (39, 40), *B.*
156 *bifidum* can induce Foxp3⁺ regulatory T cells (Tregs) (41), a subpopulation of

157 T cells fundamental in promoting and maintaining mucosal tolerance to
158 allergens (42). Mediating mechanisms of Tregs induction differ among species,
159 either via cell surface polysaccharides (*B. bifidum*, *L. reuteri*, and *B.*
160 *fragilis*) or through the production of short-chain fatty acids (SCFA)
161 (*Clostridium* sp.), resulting in the release of anti-inflammatory interleukin
162 (IL)-10. The signaling pathways of this interaction involve Toll-like
163 receptors and MyD88 signal transducer and favor the production of
164 immunoglobulin (Ig) A, which is essential to mucosal immunity and balanced
165 gut microbiota (43). Tregs specific for luminal antigens are the primary
166 negative regulators of inflammatory responses, maintaining responses of other
167 immune cells, such as Th2, within a normal range. Failure to suppress an
168 excessive Th2 response has been considered a hallmark of asthma and other
169 allergic diseases.

170 Induced Tregs are derived from the interaction of naïve T cells with antigen-
171 presenting dendritic cells (DCs) (44), which are critical regulators of T
172 cell responses and interact closely with the gut microbiome. A recent animal
173 study demonstrated that DCs produce a cytokine milieu that promotes Tregs
174 differentiation, as intraperitoneal administration of DCs reduced airway
175 inflammation in a model of allergic inflammation triggered by dust mite (45).
176 Conversely, a pro-inflammatory lipid commonly found in feces of infants at
177 risk of atopy and asthma (12,13-diHOME) reduced *in vitro* anti-inflammatory
178 cytokine secretion in human DCs (46).

179 In line with the view of commensal-induced antigen tolerance, GF mice cannot
180 be tolerized to oral antigens, have reduced levels of IL-10-producing Tregs
181 and IgA antibodies, abnormally high serum levels of the allergic marker IgE,
182 and overall phenotype characterized by a Th2 cell-biased immune response (47-
183 50). Although the susceptibility of the Th2 responses can be restored in GF
184 animals by introducing commensal bacteria, this strategy is only effective

185 when done within a narrow early life window, emphasizing the essential role
186 of microbes in immune system priming. In addition to these mechanistic
187 studies in mice, a recent study in two European longitudinal infant cohorts
188 revealed that microbiome features linked with asthma protection were
189 associated with increased tolerance to bacterial lipopolysaccharide
190 (LPS) (51), suggesting that microbiome-induced mucosal tolerance is a critical
191 mechanism of preventing allergic responses.

192 Among the molecular mechanisms that promote and maintain mucosal tolerance to
193 luminal antigens is the differentiation of induced Tregs expressing the
194 transcription factor ROR γ t in the draining lymph nodes of the small intestine
195 (52). The gut microbiota, and bacterial commensals of the order *Clostridiales*
196 and *Bacteroidales* in particular, has been reported to elicit the ROR γ t⁺ Tregs
197 induction (53, 54). Abdel-Gadir *et al.* recently showed that infants with food
198 allergy display dysbiotic fecal microbiota accompanied by decreased IgA and
199 increased IgE levels, and deficiency of ROR γ t⁺ Tregs (55). In mouse models,
200 the absence of ROR γ t⁺ Tregs results in dysregulated Th2 (53) and Th17 cell
201 responses (54). In addition, mice genetically engineered to be prone to food
202 allergy have altered gut microbiota (56) and impaired generation of allergen-
203 specific Tregs, whose function was marked by Th2-like reprogramming (57).

204 Microbiota-induced Tregs ROR γ t⁺ differentiate along a pathway that also
205 promotes Th17 immune responses (53). Several studies demonstrated that Th17
206 cells co-exist in a well-regulated balance with Foxp3⁺ Tregs, which is
207 dependent on the composition of the intestinal microbiota (58). Details of
208 how the intestinal microbiota controls the Th17 development remain unclear
209 but may involve the understudied fungal microbiota, or mycobiota (59, 60).
210 Th17 cells are abundantly present under a steady-state condition in the small
211 intestinal lamina propria where they act protectively during extracellular
212 bacterial and fungal invasion by producing pro-inflammatory cytokines IL-17

213 and IL-22. At the same time, excessive Th17 responses have been implicated in
214 lung pathogenesis in response to exogenous stimuli (61, 62).

215 In addition to modulating dendritic and T cells responses to reduce
216 inflammation and promote commensal immune reactions, the gut microbiome acts
217 on other cell types, including epithelial cells (63), basophils (64),
218 macrophages (65, 66) and innate lymphoid cells (ILC) (67). The past decade
219 has witnessed the discovery of ILC, the innate counterparts of T cells that
220 play essential roles during early life when the adaptive immunity has not
221 been fully developed (68). It is important to note that composition,
222 development, and function of ILC is regulated by the gut microbiome (69).

223 From three distinct ILC types (ILC1, ILC2, ILC3), ILC2 promote type 2
224 immunity in an antigen-independent manner and secrete IL-5 and IL-13
225 cytokines that induce eosinophilic inflammation, mucin overproduction, and
226 tissue remodeling. Experiments in mice and human cohort studies identified
227 the role of ILC2 in causing airway hyperreactivity and eosinophilic
228 inflammation and suggested that ILC2 are involved in allergic asthma
229 development and exacerbation (70-74). ILC2 have been found in intestinal
230 lamina propria as well as in circulating blood and lungs of both healthy and
231 asthmatic subjects (73), and ILC2 accumulation in airways appears to be
232 driven by cytokine IL-33 and chemokine CXCL16 in murine models of asthma
233 (75). However, parabiosis studies in which mice are surgically joined, and
234 thus develop a shared blood circulation, showed that ILC2 cells found in
235 lungs did not circulate in either steady-state conditions or inflammatory
236 conditions (76, 77), suggesting that the ILC2 accumulation in lungs mostly
237 results from the proliferation of a tissue resident ILC2 population. Still,
238 there appears to be a crosstalk between cells responsible for gut and
239 pulmonary immune homeostasis that might determine respiratory immune
240 responses to airborne allergens, irritants and respiratory viruses (78). In

241 relation to the latter, early-life respiratory viral infections are well-
242 known factor associated with an increased risk of developing childhood asthma
243 (79). The bi-directional relationship between lungs and gut is evident from
244 studies describing, for example, intestinal complications following viral
245 respiratory infection (80), oral antibiotic treatment impairing pulmonary
246 host defense (81), and commensal fungus gut colonization modulating invasive
247 fungal lung infection (60). The lung microbiome plays a vital role in
248 promoting airway tolerance (82), and alterations of lower airway microbiota
249 has been linked to the severity of airway obstruction (83). Moreover, a
250 recent study showed that microbial diversity and the relative abundances of
251 Gram-negative bacteria *Veillonella* and *Prevotella* in the airways at age one
252 month are associated with asthma by age 6 years (84). However, it remains to
253 be elucidated whether lung microbial dysbiosis drives or reflects immune
254 hyperreactivity.

255 **Emerging role of the neuroendocrine system as a key player tuning the balance**
256 **between immune system and intestinal microbiota**

257 Besides the crosstalk between ILCs and intestinal microbiome, ILCs co-
258 localize and functionally interact with cells of the enteric nervous system
259 (ENS) and neuroendocrine cells. Contained within the lamina propria, these
260 cells share a common biochemical language, consisting of cytokines,
261 chemokines, neuropeptides, neurotransmitters, hormones, and related
262 receptors, which enable them to respond to the same signals and interact with
263 each other (85). Analogous to the GALT, the ENS is the largest and most
264 complex part of the peripheral nervous system, and, unsurprisingly, the gut
265 microbiota regulates the postnatal maturation of ENS (86). Enteric glial
266 cells, the supportive cells for enteric neurons located in the lamina
267 propria, can directly modulate ILC3 cytokine release (84), sense the
268 microbiota as well as tissue damage, and respond to host-derived alarmin

269 cytokines IL-1 β and IL-33 (87). It is noteworthy that the IL-1 β and IL-33
270 have been recently shown to differentially regulate the functional adaptation
271 of Foxp3⁺ Tregs during mucosal inflammation (88).

272 In the context of asthma and other allergic diseases, neuronal regulation of
273 ILC2 can modulate the induction of type 2 inflammation. As first evidenced in
274 a murine model (89), ILC2 colocalize with adrenergic neurons in the intestine
275 and express the β 2-adrenergic receptor (β 2AR), which interacts with the
276 neurotransmitter epinephrine (adrenaline), a representative of
277 catecholamines. The same study demonstrated that β 2AR signaling suppresses
278 ILC2 proliferation, while β 2AR-deficient mice exhibited exaggerated ILC2-
279 mediated type 2 inflammation in the intestine and lungs. Thus, catecholamines
280 such as adrenaline, noradrenaline, and dopamine may have the capacity to
281 suppress ILC2 and regulate type 2 inflammation. Other β 2AR agonists, such as
282 Ventolin, have been commonly used in pulmonology as bronchodilators, the
283 first line inhaled medications used to treat asthma. From the microbiota
284 perspective, catecholamines act as signals in the gut lumen (90), and
285 noradrenaline levels in the cecal and colonic contents of specific-pathogen-
286 free mice are substantially higher than those in GF mice. Although the gut
287 microbiota can produce or stimulate the production of neurotransmitters such
288 as serotonin (91), GABA and dopamine (92), their exact contribution to the
289 levels of the neuroactive compounds remains to be determined (93). Finally,
290 catecholamines and other biogenic amine neurotransmitters are potent hormones
291 primarily released during the body's stress response (89), which has a strong
292 effect on the gut microbiome composition (94).

293 Prenatal and neonatal stress is yet another strong risk factor for asthma
294 (95). Among the biological pathways by which stress amplifies the immune
295 responses in asthma is cortisol metabolism and the hypothalamic-pituitary-
296 adrenal (HPA) axis (Figure 1), which is essential for normal neuroendocrine

297 adaptation to stress. Inflammatory mediators, including cytokines and
298 prostaglandins, are potent activators of the HPA axis (96), leading to the
299 release of glucocorticoids that have inhibitory effects on a broad range of
300 immune responses. The HPA axis dysfunction in asthma has been suggested by an
301 animal model of bronchial asthma in which exposure to early life stress
302 increased the number of eosinophils and total mononuclear cells (97). Early
303 life events program the sensitivity of the HPA axis to stress (98), and
304 multiple evidence supports the role of the gut microbiota in this process. An
305 early groundbreaking study showed that when neonatal rats are exposed to
306 bacterial LPS (endotoxin), they exhibit significantly greater hormonal
307 responses to stress, a decreased glucocorticoid feedback inhibition of the
308 HPA axis in adulthood, and reduced glucocorticoid receptor density in the
309 brain (99). Further, SCFA produced by the gut microbiota influence the
310 maturation of intestinal enteroendocrine cells and microglia, the latter
311 being cytokines releasing neuro-immune cells that activate the HPA axis. In a
312 series of animal experiments, Erny and colleagues showed that GF mice or
313 antibiotic-treated animals displayed global defects in microglia, leading to
314 impaired innate immune responses (100). The gut microbiome thus profoundly
315 impacts the normal functioning of the HPA axis that is necessary for
316 diminishing ongoing allergic reactions.

317 **The gut microbiome as a therapeutic target for atopy and asthma prevention** 318 **strategies**

319 Given the documented link between alterations of the early life gut
320 microbiome and the risk of atopy and asthma, there has been rising interest
321 in the role of probiotics, including bacterial strains of the *Lactobacillus*
322 and *Bifidobacterium* genera, for the prevention and treatment of the immune-
323 mediated disorders. However, an extensive body of research on probiotics has
324 not yet been translated into clearly defined health benefits or clinical

325 recommendations (101-103). Part of the issue is the substantial heterogeneity
326 in the strains used, their dosage, use of different prebiotics, as well as in
327 the timing and duration of the interventions among various studies. Although
328 several systematic reviews and meta-analyses showed a benefit in some
329 probiotic administrations to both mothers during pregnancy and infants in
330 their first month of life for the prevention of atopic dermatitis (104-106),
331 currently, there is not enough scientific evidence that would support a
332 general use of probiotics in the prevention of atopy and asthma.

333 Similarly, the role of breastfeeding in preventing allergic diseases has
334 gained significant attention. Breastmilk shapes the infant's gut microbiota
335 by delivering live microorganisms present in the milk and maternal skin, as
336 well as active immune factors and prebiotic oligosaccharides that affect
337 bacterial growth and metabolism. Even though there is significant discrepancy
338 regarding the effect of breastmilk on allergic diseases development (107),
339 both rodent and human studies suggest that breastmilk factors modulate
340 essential aspects of infant gut physiology, such as gut barrier function, gut
341 microbiota composition and associated metabolites production, and oral
342 tolerance induction (108-111). Variations in breastmilk immune and microbial
343 composition (112, 113), together with differences in the infant gut
344 microbiota response, can in part explain why breastfeeding seems to have an
345 inconsistent relationship with allergy and asthma prevention. For example, a
346 study of 40 mother-child dyad identified that breastmilk from mothers whose
347 children developed allergic symptoms during early childhood had lower
348 bacterial richness when compared to milk that was consumed by children
349 without the symptoms (114). Maternal lifestyle, including dietary habits and
350 physical activity, have a considerable influence on breastmilk composition,
351 as well as pre- and post-natal probiotic supplementations that can alter the
352 breastmilk microbiota composition and subsequently the infant's gut microbial

353 colonization (114, 115). A number of longitudinal birth-cohort studies
354 currently seeks to determine the effects of probiotic use on later health
355 outcomes (116, 117), still, more hypothesis-driven research is needed before
356 commencing with intervention trials in large populations. Nonetheless,
357 current findings emphasize that the immunological and microbial interactions
358 between mother and infant are critical factors in the child immune
359 development and indicate the possibility of modulating microbiota of pregnant
360 and breastfeeding women as a strategy to promote healthy gut microbial
361 colonization and normal immune maturation (111).

362 **Conclusions and future directions**

363 The balance between effector, tolerogenic, and regulatory immune mechanisms
364 relies on continuous microbial signals, especially during early life.
365 Emerging evidence suggests that infant's immune maturation is synchronized
366 with specific microbial molecules that match gradual gut colonization by
367 microbes adapted to the early life diet. Our modern lifestyle has been
368 remodeling the early life microbiome, and human birth cohort studies are
369 increasingly connecting individual microbial species with the risk of immune-
370 mediated diseases. Animal studies studying perturbations of the early-life
371 microbiome in the context of whole-body physiology will expand the
372 mechanistic understanding of the strains function and interactions with host
373 cells. Ultimately, the findings from *in vivo* models need to be translated
374 back into human trials that can inform the development of future microbiome-
375 based health interventions, for example, for asthma prevention.

376 **Key points:**

- 377 • Host-microbiome interactions in early life play a central role in
378 intestinal and pulmonary immune maturation and development, however,
379 only few functional analyses of these interactions have been described.

- 380 • Birth cohort longitudinal studies that explore details of early life
381 exposures have become instrumental in describing the bidirectional
382 relationship between the gut microbiome and the onset of allergic
383 diseases, including asthma.
- 384 • The alliance of translational microbiology, gnotobiotic animal models,
385 and high-throughput molecular approaches has become essential to
386 describe properties of individual gut microbes that might impact host
387 physiological systems and allergic diseases susceptibility.
- 388 • The use of probiotics as a prevention strategy for immune-mediated
389 diseases is currently under question and not yet fully supported by
390 scientific evidence, as the most favorable strains and their dosages,
391 together with timing and duration of the probiotic administration still
392 need to be ascertained.

393 **Acknowledgments:**

- 394 1. Acknowledgments. We thank hypothesismedia.com for creating Figure 1 and
395 members of the Arrieta lab for productive discussions.
- 396 2. Financial support and sponsorship. V.K.P is financed by the Research
397 Council of Norway FRIPRO Mobility Research Grant, which is co-funded by the
398 European Union's Seventh Framework Program for research, technological
399 development, and demonstration under Marie Curie grant. M.C.A receives
400 funding from the Canadian Institutes for Health Research, the Natural
401 Sciences and Engineering Research Council of Canada, the Cumming School of
402 Medicine at University of Calgary, The Alberta Children Hospital Research
403 Institute, the Snyder Institute of Chronic Diseases, Sick Kids Foundation,
404 the Weston Foundation and the Canadian Lung Association.
- 405 3. Conflicts of interest. None

406 **References :**

- 407 1. Gilbert JA, Lynch SV. Community ecology as a framework for human microbiome research. *Nat*
408 *Med.* 2019;25(6):884-9.
- 409 2. Bauer H, Horowitz RE, Levenson SM, Popper H. The response of the lymphatic tissue to the
410 microbial flora. *Studies on germfree mice. Am J Pathol.* 1963;42(4):471-83.
- 411 3. Gordon HA, Bruckner-Kardoss E, Staley TE, Wagner M, Wostmann BS. Characteristics of the
412 germfree rat. *Cells Tissues Organs.* 1966;64(1-3):367-89.
- 413 4. Mosconi I, Geuking MB, Zaiss MM, Massacand JC, Aschwanden C, Kwong Chung CKC, et al.
414 Intestinal bacteria induce TSLP to promote mutualistic T-cell responses. *Mucosal Immunol.* 2013;6:1157.
- 415 5. Baptista AP, Olivier BJ, Goverse G, Greuter M, Knippenberg M, Kusser K, et al. Colonic patch and
416 colonic SILT development are independent and differentially regulated events. *Mucosal Immunol.*
417 2012;6:511.
- 418 6. Kennedy EA, King KY, Baldrige MT. Mouse Microbiota Models: Comparing Germ-Free Mice and
419 Antibiotics Treatment as Tools for Modifying Gut Bacteria. *Front Physiol.* 2018;9:1534-.
- 420 7. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An Immunomodulatory Molecule of Symbiotic
421 Bacteria Directs Maturation of the Host Immune System. *Cell.* 2005;122(1):107-18.
- 422 8. Al Nabhani Z, Dulauroy S, Marques R, Cousu C, Al Bounny S, Déjardin F, et al. A Weaning
423 Reaction to Microbiota Is Required for Resistance to Immunopathologies in the Adult. *Immunity.*
424 2019;50(5):1276-88.e5.
- 425 ** This study linked alterations in the intestinal microbiota at weaning and a vigorous immune response
426 associated with a critical time window during which occurs the induction of regulatory T cells. The
427 authors also documented how a perturbation of the weaning immune reaction can result in increased
428 susceptibility to immunopathologies later in life.
- 429 9. Sonnenburg ED, Sonnenburg JL. The ancestral and industrialized gut microbiota and implications
430 for human health. *Nat Rev Microbiol.* 2019;17(6):383-90.
- 431 10. Arrieta M-C, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, et al. Early infancy
432 microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med.*
433 2015;7(307):307ra152.
- 434 11. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosch D, et al. Neonatal gut microbiota
435 associates with childhood multisensitized atopy and T cell differentiation. *Nat Med.* 2016;22:1187.
- 436 12. Arrieta M-C, Arévalo A, Stiemsma L, Dimitriu P, Chico ME, Loor S, et al. Associations Between
437 Infant Fungal and Bacterial Dysbiosis and Childhood Atopic Wheeze in a Nonindustrialized Setting. *J*
438 *Allergy Clin Immunol.* 2018;142(2):424-34.e10.
- 439 * Similarly to observations from previous USA and Canadian cohorts, the authors showed that gut
440 microbial dysbiosis of 3-months old Ecuadorian infants was associated with later development of atopic
441 wheeze. Additionally, the risk of asthma was associated with early-life antibiotic use and increased
442 relative abundance of certain gut fungi.
- 443 13. Durack J, Kimes NE, Lin DL, Rauch M, McKean M, McCauley K, et al. Delayed gut microbiota
444 development in high-risk for asthma infants is temporarily modifiable by *Lactobacillus* supplementation.
445 *Nat Commun.* 2018;9(1):707.
- 446 * The study showed that infants at high risk for asthma, exhibit a distinct meconium microbiota, delayed
447 gut microbial diversification and depletion of a range of anti-inflammatory fecal lipids. These deficits
448 could be partly rescued following *Lactobacillus rhamnosus* supplementation.
- 449 14. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an
450 ecosystem on a leash. *Nature.* 2017;548:43.

- 451 15. Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, et al.
452 Cospeciation of gut microbiota with hominids. *Science*. 2016;353(6297):380-2.
- 453 16. Pärnänen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, et al. Maternal gut
454 and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. *Nat*
455 *Commun*. 2018;9(1):3891.
- 456 17. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, et al. Mother-to-Infant Microbial
457 Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host*
458 *Microbe*. 2018;24(1):133-45.e5.
- 459 18. Korpela K, Costea P, Coelho LP, Kandels-Lewis S, Willemsen G, Boomsma DI, et al. Selective
460 maternal seeding and environment shape the human gut microbiome. *Genome Res*. 2018;28(4):561-8.
- 461 19. Korpela K, de Vos WM. Early life colonization of the human gut: microbes matter everywhere.
462 *Curr Opin Microbiol*. 2018;44:70-8.
- 463 20. Korpela K, Blakstad EW, Moltu SJ, Strømmen K, Nakstad B, Rønnestad AE, et al. Intestinal
464 microbiota development and gestational age in preterm neonates. *Sci Rep*. 2018;8(1):2453.
- 465 21. Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased
466 gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants
467 delivered by Caesarean section. *Gut*. 2014;63(4):559.
- 468 22. Gensollen T, Blumberg RS. Correlation between early-life regulation of the immune system by
469 microbiota and allergy development. *J Allergy Clin Immunol*. 2017;139(4):1084-91.
- 470 23. Martínez I, Maldonado-Gomez MX, Gomes-Neto JC, Kittana H, Ding H, Schmaltz R, et al.
471 Experimental evaluation of the importance of colonization history in early-life gut microbiota assembly.
472 *eLife*. 2018;7:e36521.
- 473 ** This experimental animal study showed that the order of species arrival and timing by which host
474 surfaces are colonized early in life has a lasting impact on the microbiome. The colonization order
475 influenced both the outcome of community assembly and the ecological success of individual colonizers.
- 476 24. Sprockett D, Fukami T, Relman DA. Role of priority effects in the early-life assembly of the gut
477 microbiota. *Nat Rev Gastroenterol Hepatol*. 2018;15:197.
- 478 25. Litvak Y, Bäumler AJ. The founder hypothesis: A basis for microbiota resistance, diversity in taxa
479 carriage, and colonization resistance against pathogens. *PLoS Path*. 2019;15(2):e1007563.
- 480 26. Yatsunenkov T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human
481 gut microbiome viewed across age and geography. *Nature*. 2012;486:222.
- 482 27. Heintz-Buschart A, Wilmes P. Human Gut Microbiome: Function Matters. *Trends Microbiol*.
483 2018;26(7):563-74.
- 484 28. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal
485 gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol*.
486 2001;107(1):129-34.
- 487 29. Raftis EJ, Delday MI, Cowie P, McCluskey SM, Singh MD, Ettorre A, et al. *Bifidobacterium breve*
488 MRx0004 protects against airway inflammation in a severe asthma model by suppressing both
489 neutrophil and eosinophil lung infiltration. *Sci Rep*. 2018;8(1):12024.
- 490 * A strain of *B. breve*, MRx0004, isolated from faeces of healthy humans, possessed a protective action
491 in a house dust mite mouse model of severe asthma.
- 492 30. Nuriel-Ohayon M, Neuman H, Ziv O, Belogolovski A, Barsheshet Y, Bloch N, et al. Progesterone
493 Increases Bifidobacterium Relative Abundance during Late Pregnancy. *Cell Rep*. 2019;27(3):730-6.e3.
- 494 31. Duranti S, Milani C, Lugli GA, Turrone F, Mancabelli L, Sanchez B, et al. Insights from genomes of
495 representatives of the human gut commensal *Bifidobacterium bifidum*. *Environ Microbiol*.
496 2015;17(7):2515-31.
- 497 32. Duranti S, Lugli GA, Milani C, James K, Mancabelli L, Turrone F, et al. *Bifidobacterium bifidum* and
498 the infant gut microbiota: an intriguing case of microbe-host co-evolution. *Environ Microbiol*. 2019;0(0).

- 499 33. Laforest-Lapointe I, Arrieta M-C. Patterns of Early-Life Gut Microbial Colonization during Human
500 Immune Development: An Ecological Perspective. *Front Immunol.* 2017;8:788-.
- 501 34. Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, et al. Association Between Breast Milk
502 Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA*
503 *Pediatrics.* 2017;171(7):647-54.
- 504 35. Neill I, Schofield Z, Hall LJ. Exploring the role of the microbiota member *Bifidobacterium* in
505 modulating immune-linked diseases. *Emerg Top Life Sci.* 2017;1(4):333.
- 506 36. Ruiz L, Delgado S, Ruas-Madiedo P, Sánchez B, Margolles A. Bifidobacteria and Their Molecular
507 Communication with the Immune System. *Front Microbiol.* 2017;8:2345-.
- 508 37. Round JL, Mazmanian SK. Inducible Foxp3⁺ regulatory T-cell development by a commensal
509 bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A.* 2010;107(27):12204.
- 510 38. Mu Q, Tavella VJ, Luo XM. Role of *Lactobacillus reuteri* in Human Health and Diseases. *Front*
511 *Microbiol.* 2018;9(757).
- 512 39. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of Colonic
513 Regulatory T Cells by Indigenous *Clostridium* Species. *Science.* 2011;331(6015):337.
- 514 40. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-
515 derived butyrate induces the differentiation of colonic regulatory T cells. *Nature.* 2013;504:446.
- 516 41. Verma R, Lee C, Jeun E-J, Yi J, Kim KS, Ghosh A, et al. Cell surface polysaccharides of
517 *Bifidobacterium bifidum* induce the generation of Foxp3⁺ regulatory T cells. *Sci Immunol.*
518 2018;3(28):eaat6975.
- 519 ** The authors showed that β -glucan/galactan polysaccharides derived from the cell wall of *B. bifidum*
520 are responsible for promoting Treg cell induction in the intestine. Further, they reported that this
521 process is dependent on intestinal dendritic cells that express Toll-like receptor 2.
- 522 42. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, et al. Intestinal
523 Tolerance Requires Gut Homing and Expansion of FoxP3⁺ Regulatory T Cells in the Lamina Propria.
524 *Immunity.* 2011;34(2):237-46.
- 525 43. Catanzaro JR, Strauss JD, Bielecka A, Porto AF, Lobo FM, Urban A, et al. IgA-deficient humans
526 exhibit gut microbiota dysbiosis despite secretion of compensatory IgM. *Sci Rep.* 2019;9(1):13574.
- 527 44. Esterházy D, Loschko J, London M, Jove V, Oliveira TY, Mucida D. Classical dendritic cells are
528 required for dietary antigen-mediated induction of peripheral Treg cells and tolerance. *Nat Immunol.*
529 2016;17:545.
- 530 45. Aragão-França LS, Rocha VCJ, Cronemberger-Andrade A, Costa FHB, Vasconcelos JF, Athanazio
531 DA, et al. Tolerogenic Dendritic Cells Reduce Airway Inflammation in a Model of Dust Mite Triggered
532 Allergic Inflammation. *Allergy Asthma Immunol Res.* 2018;10(4):406-19.
- 533 * This experimental animal study found that treatment with tolerogenic dendritic cells protects against
534 dust mite-induced allergy in a mouse model.
- 535 46. Levan SR, Stamnes KA, Lin DL, Panzer AR, Fukui E, McCauley K, et al. Elevated faecal 12,13-
536 diHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes
537 immune tolerance. *Nat. Microbiol.* 2019.
- 538 ** The authors showed that treatment with 12,13-diHOME decreased the number of regulatory T cells
539 in mice lungs and altered PPAR γ -regulated genes expression of human dendritic cells leading to reduced
540 anti-inflammatory cytokine secretion. Further, an increase in the copy number of bacterial epoxide
541 hydrolase genes among the gut microbiota or the concentration of 12,13-diHOME in infants' feces was
542 associated with a higher risk of developing atopy, eczema or asthma during childhood.
- 543 47. Cahenzli J, Köller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-
544 life colonization shapes long-term IgE levels. *Cell Host Microbe.* 2013;14(5):559-70.

545 48. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial
546 flora for the development of an IgE production system fully susceptible to oral tolerance induction. J
547 Immunol. 1997;159(4):1739.

548 49. Fritz JH, Rojas OL, Simard N, McCarthy DD, Hapfelmeier S, Rubino S, et al. Acquisition of a
549 multifunctional IgA⁺ plasma cell phenotype in the gut. Nature. 2012;481(7380):199-203.

550 50. Geuking Markus B, Cahenzli J, Lawson Melissa AE, Ng Derek CK, Slack E, Hapfelmeier S, et al.
551 Intestinal Bacterial Colonization Induces Mutualistic Regulatory T Cell Responses. Immunity.
552 2011;34(5):794-806.

553 51. Kirjavainen PV, Karvonen AM, Adams RI, Taubel M, Roponen M, Tuoresmaki P, et al. Farm-like
554 indoor microbiota in non-farm homes protects children from asthma development. Nat Med.
555 2019;25(7):1089-95.

556 ** The study investigated the well-known asthma-protective effect of farming. The authors showed that
557 the microbial composition in farm homes is distinct from that in non-farm homes. Indoor dust
558 bacterial/archaeal microbiota with similarities to farm homes appeared to be protective in non-farm
559 environments, as asthma risk decreased for children growing up in non-farm homes when their home
560 bacterial composition were more similar to farm homes. The protective effect was associated with
561 reduced proinflammatory cytokine responses against bacterial cell wall components ex vivo.

562 52. Esterházy D, Canesso MCC, Mesin L, Muller PA, de Castro TBR, Lockhart A, et al.
563 Compartmentalized gut lymph node drainage dictates adaptive immune responses. Nature.
564 2019;569(7754):126-30.

565 53. Ohnmacht C, Park J-H, Cording S, Wing JB, Atarashi K, Obata Y, et al. The microbiota regulates
566 type 2 immunity through ROR γ ⁺ T cells. Science. 2015;349(6251):989-93.

567 54. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, et al. Individual intestinal
568 symbionts induce a distinct population of ROR γ ⁺ regulatory T cells. Science. 2015;349(6251):993-7.

569 55. Abdel-Gadir A, Stephen-Victor E, Gerber GK, Noval Rivas M, Wang S, Harb H, et al. Microbiota
570 therapy acts via a regulatory T cell MyD88/ROR γ ⁺ pathway to suppress food allergy. Nat Med.
571 2019;25(7):1164-74.

572 * The authors demonstrated that gut dysbiosis promotes the breakdown of oral tolerance as a result of
573 a failure to induce protective ROR γ ⁺-dependent Treg responses in food allergic children and mice,
574 allowing instead for the emergence of Th2 cells. Administering a human-origin *Clostridiales* cluster alone
575 or together with *Subdoligranulum variabile* to mice suppressed Th2 cell activity and induced Tregs to
576 express the transcription factor ROR γ ⁺, and conferred protection to a common food allergy trigger,
577 chicken egg ovalbumin.

578 56. Noval Rivas M, Burton OT, Wise P, Zhang Y-q, Hobson SA, Garcia Lloret M, et al. A microbiota
579 signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. J
580 Allergy Clin Immunol. 2013;131(1):201-12.

581 57. Noval Rivas M, Burton Oliver T, Wise P, Charbonnier L-M, Georgiev P, Oettgen Hans C, et al.
582 Regulatory T Cell Reprogramming toward a Th2-Cell-like Lineage Impairs Oral Tolerance and Promotes
583 Food Allergy. Immunity. 2015;42(3):512-23.

584 58. Omenetti S, Pizarro TT. The Treg/Th17 Axis: A Dynamic Balance Regulated by the Gut
585 Microbiome. Front Immunol. 2015;6:639-.

586 59. Bär E, Whitney Paul G, Moor K, Reis e Sousa C, LeibundGut-Landmann S. IL-17 Regulates
587 Systemic Fungal Immunity by Controlling the Functional Competence of NK Cells. Immunity.
588 2014;40(1):117-27.

589 60. Bacher P, Hohnstein T, Beerbaum E, Röcker M, Blango MG, Kaufmann S, et al. Human Anti-
590 fungal Th17 Immunity and Pathology Rely on Cross-Reactivity against *Candida albicans*. Cell.
591 2019;176(6):1340-55.e15.

592 ** The authors demonstrated that among 30 described members of the human mycobiome, intestinal
593 *Candida albicans* is the major inducer of Th17 cells in humans and that it can influence inflammation and
594 anti-fungal responses at distal sites. By showing that the *C. albicans*-specific Th17 cells are cross-reactive
595 to other fungal species, including the airborne fungus *Aspergillus fumigatus*, the study underlined the
596 link between protective immunity in the gut and immune pathology in the lung.

597 61. De Luca A, Pariano M, Cellini B, Costantini C, Villella VR, Jose SS, et al. The IL-17F/IL-17RC Axis
598 Promotes Respiratory Allergy in the Proximal Airways. *Cell Rep.* 2017;20(7):1667-80.

599 62. Manni ML, Robinson KM, Alcorn JF. A tale of two cytokines: IL-17 and IL-22 in asthma and
600 infection. *Expert Rev Respir Med.* 2014;8(1):25-42.

601 63. Allaire JM, Crowley SM, Law HT, Chang S-Y, Ko H-J, Vallance BA. The Intestinal Epithelium:
602 Central Coordinator of Mucosal Immunity. *Trends Immunol.* 2018;39(9):677-96.

603 64. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, et al. Commensal bacteria-derived
604 signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med.* 2012;18:538.

605 65. Scott NA, Andrusaitė A, Andersen P, Lawson M, Alcon-Giner C, Leclaire C, et al. Antibiotics
606 induce sustained dysregulation of intestinal T cell immunity by perturbing macrophage homeostasis. *Sci*
607 *Transl Med.* 2018;10(464):eaa04755.

608 66. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, et al. Microbiota-
609 Dependent Crosstalk Between Macrophages and ILC3 Promotes Intestinal Homeostasis. *Science.*
610 2014;343(6178):1249288.

611 67. Cording S, Medvedovic J, Lecuyer E, Aychek T, Eberl G. Control of pathogens and microbiota by
612 innate lymphoid cells. *Microb Infect.* 2018;20(6):317-22.

613 68. Eberl G, Colonna M, Di Santo JP, McKenzie ANJ. Innate lymphoid cells: A new paradigm in
614 immunology. *Science.* 2015;348(6237):aaa6566.

615 69. Gury-BenAri M, Thaiss CA, Serafini N, Winter DR, Giladi A, Lara-Astiaso D, et al. The Spectrum
616 and Regulatory Landscape of Intestinal Innate Lymphoid Cells Are Shaped by the Microbiome. *Cell.*
617 2016;166(5):1231-46.e13.

618 70. Hong JY, Bentley JK, Chung Y, Lei J, Steenrod JM, Chen Q, et al. Neonatal rhinovirus induces
619 mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells. *J*
620 *Allergy Clin Immunol.* 2014;134(2):429-39.

621 71. Stier MT, Bloodworth MH, Toki S, Newcomb DC, Goleniewska K, Boyd KL, et al. Respiratory
622 syncytial virus infection activates IL-13-producing group 2 innate lymphoid cells through thymic stromal
623 lymphopoietin. *J Allergy Clin. Immunol.* 2016;138(3):814-24.e11.

624 72. Flayer CH, Ge MQ, Tompkins DG, Juarez M, Miller L, Royer CM, et al. Group 2 innate lymphoid
625 cells display ILC3-like functional plasticity in asthmatics and non-human primates. *J Allergy Clin Immunol.*
626 2018;141(2):AB1.

627 73. Barnig C, Cernadas M, Dutilleul S, Liu X, Perrella MA, Kazani S, et al. Lipoxin A4 regulates natural
628 killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med.* 2013;5(174):174ra26-
629 ra26.

630 74. KleinJan A, Klein Wolterink RGJ, Levani Y, de Bruijn MJW, Hoogsteden HC, van Nimwegen M, et
631 al. Enforced Expression of Gata3 in T Cells and Group 2 Innate Lymphoid Cells Increases Susceptibility to
632 Allergic Airway Inflammation in Mice. *J Immunol.* 2014;192(4):1385.

633 75. Li Y, Chen S, Chi Y, Yang Y, Chen X, Wang H, et al. Kinetics of the accumulation of group 2 innate
634 lymphoid cells in IL-33-induced and IL-25-induced murine models of asthma: a potential role for the
635 chemokine CXCL16. *Cell Mol Immunol.* 2019;16(1):75-86.

636 76. Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, et al. Interferon and IL-27
637 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. *Nat*
638 *Immunol.* 2016;17(1):76-86.

639 77. Gasteiger G, Fan X, Dikiy S, Lee SY, Rudensky AY. Tissue residency of innate lymphoid cells in
640 lymphoid and nonlymphoid organs. *Science*. 2015;350(6263):981.

641 78. Borger JG, Lau M, Hibbs ML. The Influence of Innate Lymphoid Cells and Unconventional T Cells
642 in Chronic Inflammatory Lung Disease. *Front Immunol*. 2019;10:1597-.

643 79. Toivonen L, Forsström V, Waris M, Peltola V. Acute respiratory infections in early childhood and
644 risk of asthma at age 7 years. *J Allergy Clin Immunol*. 2019;143(1):407-10.e6.

645 80. Wang J, Li F, Wei H, Lian Z-X, Sun R, Tian Z. Respiratory influenza virus infection induces
646 intestinal immune injury via microbiota-mediated Th17 cell-dependent inflammation. *J Exp Med*.
647 2014;211(12):2397.

648 81. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa e Melo F, Roelofs JJTH, de Boer JD, et al. The gut
649 microbiota plays a protective role in the host defence against pneumococcal pneumonia. *Gut*.
650 2016;65(4):575-83.

651 82. Gollwitzer ES, Saglani S, Trompette A, Yadava K, Sherburn R, McCoy KD, et al. Lung microbiota
652 promotes tolerance to allergens in neonates via PD-L1. *Nat Med*. 2014;20:642.

653 83. Turturice BA, McGee HS, Oliver B, Baraket M, Nguyen BT, Ascoli C, et al. Atopic asthmatic
654 immune phenotypes associated with airway microbiota and airway obstruction. *PLoS One*.
655 2017;12(10):e0184566-e.

656 84. Thorsen J, Rasmussen MA, Waage J, Mortensen M, Brejnrod A, Bønnelykke K, et al. Infant airway
657 microbiota and topical immune perturbations in the origins of childhood asthma. *Nat Commun*.
658 2019;10(1):5001.

659 85. Quatrini L, Vivier E, Ugolini S. Neuroendocrine regulation of innate lymphoid cells. *Immunol Rev*.
660 2018;286(1):120-36.

661 86. Kabouridis Panagiotis S, Lasrado R, McCallum S, Chng Song H, Snippert Hugo J, Clevers H, et al.
662 Microbiota Controls the Homeostasis of Glial Cells in the Gut Lamina Propria. *Neuron*. 2015;85(2):289-
663 95.

664 87. Ibiza S, García-Cassani B, Ribeiro H, Carvalho T, Almeida L, Marques R, et al. Glial-cell-derived
665 neuroregulators control type 3 innate lymphoid cells and gut defence. *Nature*. 2016;535:440.

666 88. Alvarez F, Istomine R, Shourian M, Pavey N, Al-Aubodah TA-F, Qureshi S, et al. The alarmins IL-1
667 and IL-33 differentially regulate the functional specialisation of Foxp3+ regulatory T cells during mucosal
668 inflammation. *Mucosal Immunol*. 2019;12(3):746-60.

669 89. Moriyama S, Brestoff JR, Flamar A-L, Moeller JB, Klose CSN, Rankin LC, et al. β_2 -adrenergic
670 receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science*.
671 2018;359(6379):1056-61.

672 **The study described the existence of a neuronal-derived regulatory circuit that limits group 2 innate
673 lymphoid cells (ILC2s)-dependent type 2 inflammation. This circuit includes the β_2 -adrenergic receptor
674 (β_2 AR) that ILC2s express in the small intestine, and its ligand, the neurotransmitter epinephrine. The
675 authors demonstrated that the β_2 AR pathway is a cell-intrinsic negative regulator of ILC2 responses
676 through inhibition of cell proliferation and effector function.

677 90. Bäumlér AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the
678 gut. *Nature*. 2016;535:85.

679 91. Yano Jessica M, Yu K, Donaldson Gregory P, Shastri Gauri G, Ann P, Ma L, et al. Indigenous
680 Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis. *Cell*. 2015;161(2):264-76.

681 92. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive
682 potential of the human gut microbiota in quality of life and depression. *Nat Microbiol..* 2019;4(4):623-
683 32.

684 93. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, et al. Critical role of gut
685 microbiota in the production of biologically active, free catecholamines in the gut lumen of mice.
686 *Mucosal Biology*. 2012;303(11):G1288-95.

687 94. Karl JP, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, et al. Effects of
688 Psychological, Environmental and Physical Stressors on the Gut Microbiota. *Front Microbiol.*
689 2018;9:2013.

690 95. Dreger LC, Kozyrskyj AL, HayGlass KT, Becker AB, MacNeil BJ. Lower cortisol levels in children
691 with asthma exposed to recurrent maternal distress from birth. *J Allergy Clin Immunol.* 2010;125(1):116-
692 22.

693 96. Serrats J, Schiltz JC, García-Bueno B, van Rooijen N, Reyes TM, Sawchenko PE. Dual Roles for
694 Perivascular Macrophages in Immune-to-Brain Signaling. *Neuron.* 2010;65(1):94-106.

695 97. Chida Y, Sudo N, Sonoda J, Hiramoto T, Kubo C. Early-life psychological stress exacerbates adult
696 mouse asthma via the hypothalamus-pituitary-adrenal axis. *Am J Respir Crit Care Med.* 2007;175(4):316-
697 22.

698 98. van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the Hypothalamic-Pituitary-
699 Adrenal Axis by Early Life Stress Exposure. *Front Cell Neurosci.* 2017;11:87-.

700 99. Shanks N, Larocque S, Meaney MJ. Neonatal endotoxin exposure alters the development of the
701 hypothalamic- pituitary-adrenal axis: early illness and later responsivity to stress. *J Neurosci.*
702 1995;15(1):376.

703 100. Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota
704 constantly control maturation and function of microglia in the CNS. *Nat Neurosci.* 2015;18:965.

705 101. West CE, Jenmalm MC, Kozyrskyj AL, Prescott SL. Probiotics for treatment and primary
706 prevention of allergic diseases and asthma: looking back and moving forward. *Expert Rev Clin Immunol.*
707 2016;12(6):625-39.

708 102. Wang HT, Anvari S, Anagnostou K. The Role of Probiotics in Preventing Allergic Disease. *Children*
709 (Basel, Switzerland). 2019;6(2):24.

710 103. Day RLJ, Harper AJ, Woods RM, Davies OG, Heaney LM. Probiotics: current landscape and future
711 horizons. *Future Science OA.* 2019;5(4):FSO391.

712 104. Zuccotti G, Meneghin F, Aceti A, Barone G, Callegari ML, Di Mauro A, et al. Probiotics for
713 prevention of atopic diseases in infants: systematic review and meta-analysis. *Allergy.*
714 2015;70(11):1356-71.

715 105. Cuello-Garcia CA, Brožek JL, Fiocchi A, Pawankar R, Yepes-Nuñez JJ, Terracciano L, et al.
716 Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled
717 trials. *J Allergy Clin Immunol.* 2015;136(4):952-61.

718 106. Li L, Han Z, Niu X, Zhang G, Jia Y, Zhang S, et al. Probiotic Supplementation for Prevention of
719 Atopic Dermatitis in Infants and Children: A Systematic Review and Meta-analysis. *Am J Clin Dermatol.*
720 2019;20(3):367-77.

721 107. Kim JH. Role of Breast-feeding in the Development of Atopic Dermatitis in Early Childhood.
722 *Allergy Asthma Immunol Res.* 2017;9(4):285-7.

723 108. Dogaru CM, Nyffenegger D, Pescatore AM, Spycher BD, Kuehni CE. Breastfeeding and Childhood
724 Asthma: Systematic Review and Meta-Analysis. *Am J Epidemiol.* 2014;179(10):1153-67.

725 109. Lodge CJ, Tan DJ, Lau MXZ, Dai X, Tham R, Lowe AJ, et al. Breastfeeding and asthma and
726 allergies: a systematic review and meta-analysis. *Acta Paediatr.* 2015;104(S467):38-53.

727 110. Munblit D, Verhasselt V. Allergy prevention by breastfeeding: possible mechanisms and
728 evidence from human cohorts. *Curr Opin Allergy Clin Immunol.* 2016;16(5):427-33.

729 111. van den Elsen LWJ, Garssen J, Burcelin R, Verhasselt V. Shaping the Gut Microbiota by
730 Breastfeeding: The Gateway to Allergy Prevention? *Front Pediatr.* 2019;7(47).

731 112. Moossavi S, Sepehri S, Robertson B, Bode L, Goruk S, Field CJ, et al. Composition and Variation of
732 the Human Milk Microbiota Are Influenced by Maternal and Early-Life Factors. *Cell Host Microbe.*
733 2019;25(2):324-35.e4.

734 113. Boix-Amorós A, Collado MC, Van't Land B, Calvert A, Le Doare K, Garssen J, et al. Reviewing the
735 evidence on breast milk composition and immunological outcomes. *Nutr Rev.* 2019;77(8):541-56.

736 114. Dzidic M, Mira A, Artacho A, Abrahamsson TR, Jenmalm MC, Collado MC. Allergy development is
737 associated with consumption of breastmilk with a reduced microbial richness in the first month of life.
738 *Pediatr Allergy Immunol.* 2019;n/a(n/a).

739 115. Gueimonde M, Sakata S, Kalliomäki M, Isolauri E, Benno Y, Salminen S. Effect of Maternal
740 Consumption of *Lactobacillus GG* on Transfer and Establishment of Fecal Bifidobacterial Microbiota in
741 Neonates. *J Pediatr Gastroenterol Nutr.* 2006;42(2):166-70.

742 116. Barthow C, Wickens K, Stanley T, Mitchell EA, Maude R, Abels P, et al. The Probiotics in
743 Pregnancy Study (PiP Study): rationale and design of a double-blind randomised controlled trial to
744 improve maternal health during pregnancy and prevent infant eczema and allergy. *BMC Pregnancy
745 Childbirth.* 2016;16(1):133.

746 117. Awasthi S, Wilken R, Patel F, German JB, Mills DA, Lebrilla CB, et al. Dietary supplementation
747 with *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) in healthy breastfed infants: study protocol for
748 a randomised controlled trial. *Trials.* 2016;17(1):340.

749 118. Stokholm J, Blaser MJ, Thorsen J, Rasmussen MA, Waage J, Vinding RK, et al. Maturation of the
750 gut microbiome and risk of asthma in childhood. *Nat Commun* 2018;9(1):141.

751 * As a part of investigations of the COPSAC2010, a population-based birth cohort of 700 children, the
752 study showed that one-year-old children with an immature gut microbiota composition have an
753 increased risk of asthma at age 5 years. This effect was only apparent in children born to asthmatic
754 mothers, and especially characterized an asthma phenotype also comprising allergic sensitization.

755 119. Wopereis H, Sim K, Shaw A, Warner JO, Knol J, Kroll JS. Intestinal microbiota in infants at high
756 risk for allergy: Effects of prebiotics and role in eczema development. *J Allergy Clin Immunol.*
757 2018;141(4):1334-42.e5.

758 * Infants with eczema by 18 months showed discordant development of intestinal bacterial families
759 *Enterobacteriaceae* and *Porphyromonadaceae* in the first 26 weeks, as well as decreased acquisition of
760 lactate-utilizing and butyrate-producing bacteria.

761

762 **Figure 1. Early life host-microbiome interactions influencing atopy and**
763 **asthma development.** External environmental factors shape the gut and lung
764 microbiome in early life and can cause perturbations that lead to immune-
765 mediated allergic diseases such as atopy and asthma. Gut microbiome
766 composition can dictate susceptibility to allergen-specific responses, which
767 are a result of interactions between microbial molecules and immune cells.
768 Perturbations of the early life microbiome might mediate alterations in the
769 number of regulatory T cells (Tregs), type 2 helper (Th2) and Th17 cells, as
770 well as in the cytokine and antibody milieu. Changes that have been
771 associated with asthmatic immune phenotype are indicated, including
772 increase/decrease of specific interleukins (IL) and immunoglobulins (Ig).
773 Microbiome-derived signals also impact the neuroendocrine system, which is
774 capable of modulating immune mechanisms in allergic responses via the
775 hypothalamic-pituitary-adrenal (HPA) axis and dysregulation of cortisol
776 release. White arrows indicate adrenal gland on top of the kidney and
777 hypothalamus located in the brain above the pituitary gland. Microbiome
778 composition can also influence the activities of microglia (Glia), neurons
779 and neuroendocrine cells (NECs), which are known to interact with immune
780 cells relevant in the pathogenesis of asthma.

781

1 **Table 1** Prospective birth cohort studies combining microbiome analysis and determination of atopy and

2 asthma risk ¹.

Authors & Years	Main objectives(s)	Birth Cohort Acronym	Study Population ²	Samples Collected	Techniques to assign bacterial and fungal taxa, and for metabolite detection	Key findings	Reference
Kirjavainen et al. 2019	To identify microbial exposures that could be exploited for preventive interventions of asthma.	LUKAS1&2, GABRIELA	Children ≤ 6 years, N=395+1031	Living room floor dust samples	16S rRNA gene and ITS region amplicon sequencing	By modeling differences in house dust microbiota between farm and non-farm homes of Finnish families, the authors showed that in children growing up in non-farm homes, asthma risk decreases when their home bacterial composition is more similar to farm homes.	(51)
Levan et al. 2019	To determine whether elevated faecal concentrations of 12,13-diHOME promote allergic inflammation by inducing DCs dysfunction, resulting in a subsequent reduction in the number of anti-inflammatory Treg cells.	Subsets of the WHEALS and TIPS cohorts	Infants 1 month old, N=41+50	Stool	Shotgun metagenomic sequencing, LC-MS metabolomic analyses	Increase in the copy number of bacterial epoxide hydrolase genes among the gut microbiota or the concentration of 12,13-diHOME in infants feces, was associated with an increased probability of developing atopy, eczema or asthma during childhood.	(46)
Arrieta et al. 2018	To explore whether similar microbiome patterns (as observed in Canada) can be observed in a geographically distinct population with similar reported rates of asthma prevalence to Canada.	ECUAVIDA	Infants 3 months old N=97	Stool	16S rRNA gene and 18S region amplicon sequencing, LC/MS metabolomic analyses	Microbial dysbiosis in 3 months-old Ecuadorian infants was associated with later development of atopic wheeze. The dysbiosis was characterized by abundance changes in several bacterial taxa (<i>Streptococcus</i> sp., <i>Bacteroides</i> sp., <i>Ruminococcus gnavus</i> , <i>Bifidobacterium</i>) and increase in relative abundance of fungi <i>Pichia kudriavzevii</i> . Levels of the fecal short-chain fatty acids acetate and caproate were reduced and increased, respectively, in the stool samples of children who went on to have atopic wheeze.	(12)
Durack et al. 2018	To determine whether neonates at high risk for asthma exhibit meconium gut microbiota dysbiosis and a reduced rate of gut bacterial diversification over the first year of life.	Subset of TIPS and DIMES cohorts	Infants ≤ 12 months old, N=25+29	Stool	16S rRNA gene amplicon sequencing, LC/MS metabolomic analyses	Children at high risk for asthma, exhibited a distinct meconium microbiota, delayed gut microbial diversification and were depleted for a range of anti-inflammatory fecal lipids in infancy. These deficits were partly rescued by <i>Lactobacillus rhamnosus</i> supplementation. However, this effect was lost after cessation of the supplementation.	(13)
Stokholm et al. 2018	To analyze the nature of gut colonization patterns during the first year of life, and the associations of these patterns with the later risk of asthma.	COPSAC ₂₀₁₀	Children ≤ 5 years, N=690	Stool	16S rRNA gene amplicon sequencing	One-year-old children with an immature gut microbiota composition had an increased risk of asthma at age 5 years. This effect was only apparent in children born to asthmatic mothers, and especially characterized an asthma phenotype also comprising allergic sensitization.	(118)

Wopereis et al. 2018	To investigate the effects of interventions and breast-feeding on fecal microbiota. Additionally, to identify microbial patterns associated with the onset of eczema.	PATCH	Infants in the first 26 weeks N=138	Stool	16S rRNA gene amplicon sequencing	Infants with eczema by 18 months showed discordant development of bacterial genera of <i>Enterobacteriaceae</i> and <i>Parabacteroides</i> species in the first 26 weeks, as well as decreased acquisition of lactate-utilizing bacteria producing butyrate.	(119)
Fujimura et al. 2016	To investigate whether compositionally distinct human neonatal gut microbiota exist and is differentially related to relative risk of childhood atopy and asthma.	Subset of WHEALS	Infants 1-11 months N=97	Stool	16S rRNA gene amplicon sequencing	American infants at risk of asthma showed lower relative abundance of certain bacteria (<i>Bifidobacterium</i> , <i>Akkermansia</i> and <i>Faecalibacterium</i>), higher relative abundance of particular fungi (<i>Candida</i> and <i>Rhodotorula</i>) and a distinct fecal metabolome enriched for pro-inflammatory metabolites. <i>Ex vivo</i> culture of human adult peripheral T cells with sterile fecal water from infants having a high risk of asthma increased the proportion of CD4+ cells producing interleukin (IL)-4 and reduced the relative abundance of CD4+CD25+FOXP3+ cells.	(11)
Arrieta et al. 2015	To elucidate the factors involved in asthma and atopic disease development.	Subset of CHILD	Infants 3- and 12-months old N=312	Stool	16S rRNA gene amplicon sequencing	The study for the first time reported that infants at risk of asthma have transient gut microbial dysbiosis during the first 100 days of life with significantly decreased relative abundance of the bacterial genera <i>Lachnospira</i> , <i>Veillonella</i> , <i>Faecalibacterium</i> , and <i>Rothia</i> . The reduction in bacterial taxa was accompanied by reduced levels of fecal acetate and dysregulation of enterohepatic metabolites.	(10)

3 ¹ Abbreviations used: ITS - internal transcribed spacer, LC-MS - liquid chromatography mass spectrometry,

4 12,13-diHOME - 12,13-dihydroxy-9Z-octadecenoic acid, DCs - Dendritic cells

5 ² Multiple numbers refer to the listed birth cohorts respectively.

6

7

Figure

MICROBIOME COMPOSITION

Negative external factors affecting gut and lung microbiome composition

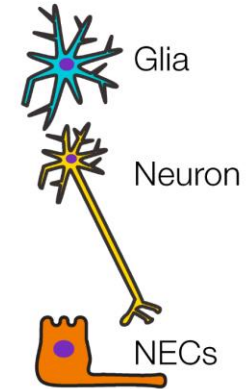
- Antibiotic use
- Caesarean section
- Bottle-feeding
- Urban living
- Dysbiotic maternal microbiome



NEURO-ENDOCRINE SYSTEM

Hormones and neurotransmitters

- Norepinephrine
- Epinephrine
- Cortisol
- Glucocorticoids
- Prostaglandins



Biomolecules trafficking via circulatory systems

IMMUNE SYSTEM

Cytokine and antibody levels

