



The evolution of the hygiene hypothesis: the role of early-life exposures to viruses and microbes and their relationship to asthma and allergic diseases

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Purpose of review

Understanding the mechanisms involved in the development of asthma and allergic diseases is expanding, due in part to sequencing advances that have led to the identification of new viral strains such as human rhinovirus strain C (HRV-C) and the human microbiome project.

Recent findings

Recent studies have identified new ways in which viral and microbial exposures in early life interact with host genetic background/variants to modify the risk for developing asthma and allergic diseases. Recent research suggests that HRV-C is the main pathogenic agent associated with infant wheeze, hospitalizations and likely the subsequent development of asthma. Pulmonary ³He MRI suggests that HRV infection in early childhood and subsequent immune responses initiate airway remodeling. Numerous studies of the microbiome indicate that intestinal and airway microbiome diversity and composition contribute to the cause of asthma and allergic diseases.

Summary

Susceptibility to asthma and allergic diseases is complex and involves genetic variants and environmental exposures (bacteria, viruses, smoking, and pet ownership), alteration of our microbiome and potentially large-scale manipulation of the environment over the past century.

Keywords

allergies, asthma, human rhinovirus, microbiome, viruses

INTRODUCTION

Epidemiologists have long sought to understand the factors involved in the initiation and exacerbation of asthma. Epidemiological studies clearly indicate that asthma is a complex disease resulting from a combination of genetic susceptibility, environmental exposures and their interactions. There is compelling evidence from birth cohort studies that early-life exposures such as siblings (birth order), dust, microbes, pets and dust from farm animals [1–5] are protective, whereas exposures such as urban residence, smaller family size, Cesarean section births and use of antibiotics during pregnancy may result in an increased risk for asthma and allergic diseases [6,7,8^{*}]. Environmental exposures can cause epigenetic changes through DNA methylation, histone acetylation, and micro-RNA changes, resulting in an altered immune response to microbes [9,10]. With the completion of multiple genome-wide association studies (GWAS) across the globe, numerous genes have been associated with asthma and related phenotypes, and several genes

including genes in the *GSDMB/ORMDL3* region (17q12-q21 region) [11], *NFKBI*, *IL1R2*, *LBP*, *IL18RAP* and *TLR1* have been reported to interact with viral infections in the first year of life to modify the risk for asthma and allergic diseases [2,12^{**},13^{**}]. This review will focus on findings reported in the past year that provide insight into the capacity for viral infections and microbes to modulate the human immune system. Emerging research is providing clues for the mechanisms whereby genetic susceptibility and environmental exposures relate

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KEY POINTS

- New evidence suggests that not all HRV strains are equally pathogenic and HRV-C may be the causative agent for asthma exacerbations and/or susceptibility.
- New evidence suggests that HRV infection and subsequent immune responses may initiate airway remodeling.
- The intestinal and airway microbiome diversity and composition contribute to the cause of asthma and allergic diseases.

and interact for the cause of asthma and allergic diseases.

THE HYGIENE HYPOTHESIS

The 'hygiene hypothesis' was initially introduced by Strachan [14] in 1989 to explain why asthma and allergic disorders were increasing in industrialized countries. Changes in the microbial environment influence how the human immune system develops. Early-life exposures to viral and bacterial organisms and their products (e.g. endotoxins) 'shape' and train the immune system, and elimination of these exposures results in a shift towards an inflammatory immune response associated with asthma and allergic diseases. Increase in asthma and allergy has also been accompanied by an increase in autoimmune diseases (1950–2000), and these observations correspond with a decrease in infectious diseases [15]. This leads to the hypothesis that in the absence of foreign invaders, the immune response in individuals with 'alert/vigilant' immune systems, which served as a survival advantage in a world abundant with microbes and viruses, attacks itself in the absence of these foreign invaders resulting in increases in autoimmune diseases such as asthma.

One possible mechanism to explain the 'hygiene hypothesis' is that exposure to microbial pathogens and animals in infancy prevents atopy by boosting T_H1 -like cytokine responses or by modifying T_H2 -like immune responses [4,16–24]. Atopy in adults is characterized by production of T_H2 cytokines such as interleukin (IL)-4 and IL-5, which promote immunoglobulin E (IgE) production and eosinophil recruitment that are characteristic of the allergic inflammatory response; nonatopic individuals show a predominantly T_H1 immune response. These responses start *in utero*; samples taken from infant cord blood show a predominant T_H2 profile [18]. Lack of exposure to bacteria in early infancy

locks the immune system into the predominantly T_H2 state that is seen in atopic individuals [18,19,25,26] and viral infections that are associated with wheezing increase the risk for developing asthma [13²²,27²²].

THE HUMAN MICROBIOME AND THE MICROBIOTA HYPOTHESES

The human microbiome has changed and evolved with the human species, and recent studies indicate that the microbiome plays a crucial role in the development of the immune system. Factors that alter or change the composition and diversity of the microbiome, such as the method of infant delivery or usage of antibiotics, have a strong effect on immune responses, altering the immune response from one that is consistent with a state of tolerance to a dysfunctional hyper-responsive state that is associated with asthma and allergic diseases. The concept that the microbiome may modify the risk of asthma and allergic diseases is by no means novel. Infection with *Helicobacter pylori* is associated with a decreased risk of asthma, and allergic diseases and infection is more common in rural settings [28²²,29–31]. As infection with *H. pylori* is also associated with gastritis, stomach ulcers and cancer, antibiotic treatment for the infection has been implemented [32]. Antibiotic effects are not limited to *H. pylori*, but affect the entire microbiome. The 'disappearing microbiota' hypothesis postulates that changes in the way humans interact with microbes and their environment have resulted in decreased exposure to microbes and animals. Changes in lifestyle, such as movement from rural to urban living environments, intervention with antibiotics and other effects of industrialization, have altered the ways humans interact with animals and microbes, changing our microbiome, resulting in an increased prevalence of asthma and allergic diseases in industrialized countries.

Our ability to evaluate the microbiome and test the microbiota hypotheses has until recently been limited, but advancements in sequencing technologies and the large-scale human microbiome project (HMP) that was initiated by the National Institutes of Health in 2007 [33] have resulted in the initiation of new studies to evaluate these hypotheses. While the HMP is encompassing and is evaluating all human orifices and mucosal bodies, this review will focus on the respiratory and intestinal microbiome.

THE BIODIVERSITY HYPOTHESIS

The 'biodiversity' hypothesis postulates the loss of biodiversity due to climate change, and human

activity correlates with an increasing prevalence of inflammatory diseases [34^{••}] such as asthma and allergic diseases. The protective effect of the farm environment on the development of asthma and allergies is likely due to the diversity of exposure to microorganisms [35] to a developing neonatal immune system. The hypothesis that ‘biodiversity’ and microbial-rich environments confer protection against asthma and allergic diseases is consistent and complementary to the ‘hygiene hypothesis’, the ‘disappearing microbiota’, and results from the HMP, linking together these complementary and converging hypotheses. For a more in-depth look and review of each of these hypotheses, the reader is referred to several outstanding review papers that have recently been published [34^{••},36^{••},37^{••},38[•]].

EVOLUTION OF THE HYGIENE HYPOTHESIS

All of these hypotheses (hygiene, disappearing microbiota, and biodiversity) are complementary and intersecting. Just as humans evolved, so has the hygiene hypothesis. This review will cover many of the papers published in 2013 that are informing our understanding of the hygiene hypothesis. Given the large volume of research and numerous high-quality papers that have been published, it is not possible to review all papers, nor is this the intent of this review. The focus rather will be on studies that have informed the hygiene hypothesis and are informing us on the mechanisms for gene–environment interaction.

VIRAL INFECTIONS

Viral infections are well recognized as risk factors for asthma [39–45] and may cause up to 60% of asthma exacerbations [46], although it is not clear if the viruses are causal, impacting and modifying the growth and development of the immune system, or if viral infection agitates and initiates symptoms in a genetically susceptible individual with impaired lung function or a combination of the two [44,47]. The ‘two-hit’ hypothesis has been postulated, whereby viral infections promote asthma mainly in children already predisposed to getting asthma [44]. Clinically it has been observed that children with atopic disease have more severe viral infections and allergic sensitization precedes wheezing with respiratory infection [48[•],49,50[•],51]. This observation is supported by a recent study [52] that examined the serum of 287 asthmatic children and demonstrated increased levels of serum titers for allergen-specific IgE, increasing the probability of wheezing with human rhinovirus (HRV) infection.

Numerous viruses have been associated with asthma susceptibility, the two viruses most frequently associated are respiratory syncytial virus (RSV), and HRV, which is a member of the Picornavirus family [53[•]]. HRV is the primary virus responsible for the common cold. Infection with RSV in infants and children results in symptoms that range from absent to those hospitalized with severe respiratory distress [48[•]]. Many early studies focused on RSV as the primary candidate for asthma susceptibility and exacerbation. More recent research has shown that wheezing episodes in response to HRV infection have a stronger association than RSV with future wheezing and asthma in early life [27^{••}]. Several genes including genes in the *GSDMB/ORMDL3* region (17q12-q21 region) – *NFKBI*, *IL1R2*, *LBP*, *IL18RAP* and *TLR1* – have been reported to interact with viral infections to modify the risk of asthma and allergic diseases [2,11,12^{••},13^{••}]. Recently, the Canadian Asthma Primary Prevention Study (CAPPS) – a high-risk birth cohort that recruited mothers in the second and third trimester of pregnancy and followed children from birth to age 7, evaluated viral infections in the first 2 years of life and interactions with genetic polymorphisms. The study [2] examined genetic variants in genes involved in innate immune response and viral infections in the first year of life; they found the most significant interactions between genetic variants in *TLR2* and *IL1R2* × picornavirus virus infection and risk for atopic asthma, *LBP* × picornavirus infection and airway hyper-responsiveness (AHR), *IL18RAP* and *TLR* × parainfluenza virus infection and AHR, and *NFKBIB* × RSV and AHR. This is one of the several recently published studies that implicate early viral infection in the genetically susceptible child in the cause of asthma and allergic diseases.

The Childhood Origin of Asthma (COAST) and the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) birth cohorts reported that 17q21 variations were associated with HRV wheezing illness in early life, but not with RSV [11]. Additional investigation demonstrated increased expression of *IKZF3*, *GSDMB*, and *ORMDL3* in peripheral blood mononuclear cells stimulated with HRV. This finding is intriguing, because HRV exposure and infection are so common/ubiquitous. The mean age of a first symptomatic respiratory viral infection is 4–6 months of age and virtually all children (>90%) are exposed by the age of 2 [48[•]]. It was first reported in 2010 that viral infection interacted (effect modification) with variants in the 17q21 region [11]. Given the ubiquitous exposure to HRV, the relationship to asthma susceptibility and exacerbation can likely be explained by the genetic background of

the virus and host, and/or host–viral interaction. Caliskan *et al.* [13²²] determined that the effects of HRV infection can be stratified by wheezing and genetic susceptibility. A recent study by Cox *et al.* [27²²] indicates that there may be more to this story; prior to 2007, there were two species of HRV (A and B); improvements in detection identified a new class HRV-C. Cox *et al.* [27²²] reported that in children presenting to the emergency department with acute wheezing and a viral infection, HRV was the most common virus (68.5%), and HRV-C was the most common HRV species identified. Furthermore, children with HRV-C infection had higher risk for subsequent hospital admissions and a higher proportion of emergency room visits prior to recruitment when compared with children with infection with HRV-A and HRV-B. The greatest risk for subsequent hospitalization was in atopic individuals infected with HRV-C. This demonstrated that the HRV-C viral class is associated with severity symptoms and risk for hospitalization, and even stronger associations were observed in atopic children. The question of whether HRV-C infection is causative in the development of asthma or is an indicator of a susceptible child is unknown. It has been suggested that HRV-related wheezing could be likened to a positive ‘stress test’ that identifies those children at the greatest risk for persistent asthma [54²²]. Furthermore, the mathematical models of Adler and Kim [55²²] predicts the possibility of highly damaging viruses, which could be used to describe HRV-C and model more severe rhinovirus infection.

There is increasing evidence that inherited variation in innate immune response genes may interact with viral infection to predispose to disease [2,12²²]. Ali *et al.* [12²²] used a systems biology approach to demonstrate that *NFKBIA* is a central hub in transcriptional responses of childhood lung disease including RSV infection, asthma and bronchopulmonary dysplasia, and that genetic variation in the promoter of *NFKBIA* is associated with differential susceptibility to severe bronchiolitis, AHR, and severe bronchopulmonary dysplasia following infection with RSV. This indicated that early viral infections in a genetically susceptible child are likely involved in disease susceptibility, which involves multiple genetic variants and viruses.

Viral illnesses result in increased mucus production that can lead to complete occlusion of the small airways, leading to ventilation and perfusion abnormalities that are present as early as 2–3 years of life [56²²]. Cadman *et al.* [56²²] hypothesized that a child’s lung function and prior history of viral illness would be associated with regional patterns of airway obstruction at age 9 and 10 years in children from the COAST study.

Investigators used pulmonary ³He MRI, which has been used successfully to detect abnormalities in lung ventilation and structure in adult asthmatic patients to evaluate a subset of children from the COAST study. MRI results demonstrated more and larger regions of ventilation defect and greater degree of restricted gas diffusion in children with asthma compared with those seen in children without asthma, indicating that viral infection and subsequent immune responses may play a role in the airway remodeling that occurs in asthmatic children. These observations are further supported by data from a mouse model of asthma which observed that a progression to an asthmatic phenotype was associated with epigenetic regulation of genes associated inflammation and structural remodeling [57²²]. Results from animal studies indicate that responses to mucosal infection surfaces in early life are protective against the disease, suggesting that properties of neonatal immune cells and subsequent maturation of the immune system may be regulated by epigenetic mechanisms [58²²]. It is believed that DNA methylation, which is one form of epigenetic regulation, originated as a host cell defense mechanism, helping to keep integrated viral sequences in a repressed state, suppressing viral expression in the infected cells [59]. DNA methylation plays a crucial role in differentiation of immune cells and regulation of cell-specific gene expression in the immune system [60]. Because of the importance of DNA methylation in the development of immune cells and because aberrant DNA methylation has already been associated with several immune deficiencies and autoimmune disorders, it is a promising target and potential biomarker for other immune disorders such as asthma [60], and viral infections may be the catalyst for epigenetic changes in gene expression and airway remodeling.

CONTRIBUTIONS FROM THE MICROBIOME

Differences in airway microbial communities are present in adult asthma [61] and can be altered by vitamin D and probiotics [62,63²²]. Reduced diversity of the intestinal microbiota in infancy may play an important role in the development of childhood allergies and asthma due the substantial effect of the flora on mucosal immunity [63²²,64,65²²]. Support for this hypothesis increased with findings that low total diversity of the gut microbiota during the first month of life was associated with eczema [9,15,46,66] and allergen sensitization [9,15,46,66], and more recently asthma at 7 years of age [65²²]. Abrahamsson *et al.* [65²²] evaluated 47 children (20 with IgE-associated eczema and 27 without any

allergic disease) from a Swedish birth cohort study, initiated to evaluate the efficacy of probiotic supplementation until 2 years of life for the primary prevention of asthma and allergic diseases. Stool samples were collected from infants at 5–7 days, 1 month and 1 year of age. Shannon diversity index, which accounts for both abundance and evenness of the species diversity in a community, was used to characterize the gut microbiome. Investigators found an association with lower diversity at both 1 week and 1 month of age, and subsequent risk for doctor-diagnosed asthma at 7 years of age. There was no association between diversity of the gut microbiome at 1 year of age and asthma, suggesting that effects on immune development are established early in infancy: findings consistent with the protective effects of farms and infant exposure [67–69]. This is an initial study linking low diversity of gut microbiota in the first year of life to asthma; the study is small and the findings need confirmation. Associations between early-life microbiota and the risk of asthma have not been well investigated to date, likely due to the length of follow-up required (~7 years) to evaluate asthma associations.

A meta-analysis to evaluate the effects of probiotic supplementation in infants [63²²] was published in 2013. The analysis concluded that probiotic supplementation significantly reduced the risk of atopic sensitization [odds ratio (OR) 0.88, $P=0.035$], but may not reduce the risk of asthma.

Results from infant meconium in a Spanish birth cohort [70²³] indicate that the meconium microbiota has an interuterine origin and participates in gut colonization in the infant. In the small number of infants studied ($n=20$), meconium samples clustered into two types with different bacterial diversity and composition. In the less diverse type, they found an enteric bacterial infection that was associated with maternal atopic eczema ($P=0.03$), and the second type was dominated by lactic acid bacteria and was associated with infant respiratory problems ($P=0.04$). Results from investigation of the intestinal microbiome in infants from the Canadian Healthy Infant Longitudinal Development Study (CHILD) [71²⁴] demonstrate that there are differences in both the types and abundance of bacteria in infant households with pets and older siblings. Both birth order (lower in the birth order the better) [5,14,72] and dog ownership [3,73] are known to have protective effects, and results from the CHILD study suggest that modification of the infant gut microbiome may be a possible mechanism for these protective effects. Given that dog ownership is both a modifiable and protective factor, in the future, families with genetic susceptibility may be advised to bring home a dog with their newborn or to

institute probiotic supplementation. These are just two examples of easily accessible, low-cost interventions that could be implemented on population level to reduce the prevalence of asthma and allergic diseases that are being informed by recent genetic and microbiome studies.

AIRWAY MICROBIOME

The role of the airway microbiome in response to corticosteroids in asthmatic patients was investigated in 39 individuals with asthma, 29 of whom were resistant to corticosteroids and 10 responsive patients [74²⁵]. Researchers found that the microbiome did not differ in diversity or composition at the phylum level (i.e. bacterial families), but did differ at the genus level, with distinct genus expansion in 14 patients with corticosteroid asthma.

CONCLUSION

The cause of asthma and allergic diseases is complex with many interacting components. However, thanks in large part to recent advances in sequencing technology our understanding of the mechanisms involved is slowly evolving. Studies are elucidating that susceptibility involves genetic variants and environmental exposures, alteration of our microbiome and potentially large-scale manipulation of the environment over the past century. Many of the findings reviewed are preliminary and will need confirmation, but exciting new paradigms and hypotheses are emerging as a result of these preliminary findings.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Ege MJ, Strachan DP, Cookson WO, *et al.* Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol* 2011; 127:138–144 (44 e1–4).
2. Daley D, Park JE, He JQ, *et al.* Associations and interactions of genetic polymorphisms in innate immunity genes with early viral infections and susceptibility to asthma and asthma-related phenotypes. *J Allergy Clin Immunol* 2012; 130:1284–1293.

3. Gern JE, Reardon CL, Hoffman S, *et al.* Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol* 2004; 113:307–314.
 4. Riedler J, Braun-Fahrlander C, Eder W, *et al.* Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; 358:1129–1133.
 5. Upchurch S, Harris JM, Cullinan P. Temporal changes in UK birth order and the prevalence of atopy. *Allergy* 2010; 65:1039–1041.
 6. Majkowska-Wojciechowska B, Pelka J, Korzon L, *et al.* Prevalence of allergy, patterns of allergic sensitization and allergy risk factors in rural and urban children. *Allergy* 2007; 62:1044–1050.
 7. Almquist C, Cnattingius S, Lichtenstein P, Lundholm C. The impact of birth mode of delivery on childhood asthma and allergic diseases: a sibling study. *Clin Exp Allergy* 2012; 42:1369–1376.
 8. Stensballe LG, Simonsen J, Jensen SM, *et al.* Use of antibiotics during pregnancy increases the risk of asthma in early childhood. *J Pediatr* 2013; 162:832–838 (e3).
- One of the first publications to evaluate antibiotic usage in pregnancy and subsequent risk for childhood asthma in the child.
9. Abrahamsson TR, Jakobsson HE, Andersson AF, *et al.* Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012; 129:434–440 (40 e1–2).
 10. Durham AL, Wiegman C, Adcock IM. Epigenetics of asthma. *Biochim Biophys Acta* 2011; 1810:1103–1109.
 11. Smit LA, Bouzigon E, Pin I, *et al.* 17q21 variants modify the association between early respiratory infections and asthma. *Eur Respir J* 2010; 36:57–64.
 12. Ali S, Hirschfeld AF, Mayer ML, *et al.* Functional genetic variation in NFKBIA and susceptibility to childhood asthma, bronchiolitis, and bronchopulmonary dysplasia. *J Immunol* 2013; 190:3949–3958.
- Using a systems biology approach authors demonstrated that NFKBIA/Ikappa Balpha is a central hub in transcriptional responses of prevalent childhood lung diseases, including respiratory syncytial virus infection, asthma, and bronchopulmonary dysplasia. They further demonstrated that genetic variation in the promoter of NFKBIA is associated with differential susceptibility to severe bronchiolitis following infection with respiratory syncytial virus, airway hyperresponsiveness, and severe bronchopulmonary dysplasia.
13. Caliskan M, Bochkov YA, Kreiner-Moller E, *et al.* Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 2013; 368:1398–1407.
- Genetic variants in the region of 17q21 are associated with HRV wheezing illnesses in early childhood providing further evidence that HRV infection is associated with asthma susceptibility in genetically predisposed children. Furthermore, mounting evidence suggests that the 17q21 genetic variants interact with environmental exposures such as environmental tobacco smoke and HRV infection, modifying gene expression in the region.
14. Strachan DP. Hay fever, hygiene, and household size. *Br Med J* 1989; 299:1259–1260.
 15. Wang M, Karlsson C, Olsson C, *et al.* Reduced diversity in the early fecal microbiota of infants with atopic eczema. *J Allergy Clin Immunol* 2008; 121:129–134.
 16. Rook GA, Stanford JL. Skin-test responses to mycobacteria in atopy and asthma. *Allergy* 1999; 54:285–286.
 17. Von Ehrenstein OS, Von Mutius E, Illi S, *et al.* Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000; 30:187–193.
 18. Prescott SL, Macaubas C, Holt BJ, *et al.* Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; 160:4730–4737.
 19. Prescott SL, Macaubas C, Smallacombe T, *et al.* Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353:196–200.
 20. Platts-Mills TA, Vaughan JW, Blumenthal K, *et al.* Serum IgG and IgG4 antibodies to Fel d 1 among children exposed to 20 microg Fel d 1 at home: relevance of a nonallergic modified Th2 response. *Int Arch Allergy Immunol* 2001; 124:126–129.
 21. Platts-Mills T, Vaughan J, Squillace S, *et al.* Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357:752–756.
 22. Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med* 2001; 164 (8 Pt 2):S1–S5.
 23. Platts-Mills TA, Vaughan JW, Blumenthal K, *et al.* Decreased prevalence of asthma among children with high exposure to cat allergen: relevance of the modified Th2 response. *Mediators Inflamm* 2001; 10:288–291.
 24. Platts-Mills TA, Woodfolk JA, Sporik RB. Con: the increase in asthma cannot be ascribed to cleanliness. *Am J Respir Crit Care Med* 2001; 164:1107–1108.
 25. Baldini M, Lohman IC, Halonen M, *et al.* A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; 20:976–983.
 26. Baldini M, Vercelli D, Martinez FD. CD14: an example of gene by environment interaction in allergic disease. *Allergy* 2002; 57:188–192.
 27. Cox DW, Bizzintino J, Ferrari G, *et al.* Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. *Am J Respir Crit Care Med* 2013; 188:1358–1364.
- Asthma exacerbations and susceptibility are associated with infection with the HRV virus, but not all strains of HRV may be pathogenic. Evidence is presented that infection with the HRV-C strain is more pathogenic and associated with wheezing and hospitalizations than A or B strains. Furthermore, effects of infection with HRV-C are amplified in children with atopy.
28. Zhou X, Wu J, Zhang G. Association between *Helicobacter pylori* and asthma: a meta-analysis. *Eur J Gastroenterol Hepatol* 2013; 25:460–468.
- Meta-analysis of 14 studies, involving 28 283 patients, found a significantly lower rate of *H. pylori* infection in the asthmatic patients than in the controls [OR 0.84, 95% confidence interval (CI) 0.73–0.96, *P* = 0.013].
29. Taube C, Muller A. The role of *Helicobacter pylori* infection in the development of allergic asthma. *Expert Rev Respir Med* 2012; 6:441–449.
 30. Zevit N, Balicer RD, Cohen HA, *et al.* Inverse association between *Helicobacter pylori* and pediatric asthma in a high-prevalence population. *Helicobacter* 2012; 17:30–35.
 31. Reibman J, Marmor M, Filner J, *et al.* Asthma is inversely associated with *Helicobacter pylori* status in an urban population. *PLoS One* 2008; 3:e4060.
 32. Marshall B. *Helicobacter pylori*: a nobel pursuit? *Can J Gastroenterol* 2008; 22:895–896.
 33. McGuire AL, Colgrove J, Whitney SN, *et al.* Ethical, legal, and social considerations in conducting the Human Microbiome Project. *Genome Res* 2008; 18:1861–1864.
 34. Haahtela T, Holgate S, Pawankar R, *et al.* The biodiversity hypothesis and allergic disease: world allergy organization position statement. *World Allergy Organ J* 2013; 6:3.
- Review paper which provides a nice overview of the biodiversity hypothesis.
35. Ege MJ, Mayer M, Normand AC, *et al.* Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011; 364:701–709.
 36. Bendiks M, Kopp MV. The relationship between advances in understanding the microbiome and the maturing hygiene hypothesis. *Curr Allergy Asthma Rep* 2013; 13:487–494.
- Recommended review paper for intersection between the microbiome and how it is informing our understanding of the 'hygiene hypothesis'.
37. Brown EM, Arrieta MC, Finlay BB. A fresh look at the hygiene hypothesis: how intestinal microbial exposure drives immune effector responses in atopic disease. *Semin Immunol* 2013; 25:378–387.
- Recommended review paper examining the hygiene hypothesis and how the intestinal microbiome modifies immune response. Reviews the mechanisms involved in the immune response and interactions with the intestinal microbiome.
38. Gann H, Neves JF, Blumberg RS, Renz H. Effect of barrier microbes on organ-based inflammation. *J Allergy Clin Immunol* 2013; 131:1465–1478.
- Review paper that evaluates the microbiome and immune responses with a focus on inflammation and immunological responses.
39. Le Souef PN. Gene-environmental interaction in the development of atopic asthma: new developments. *Curr Opin Allergy Clin Immunol* 2009; 9:123–127.
 40. Sly PD. The early origins of asthma: who is really at risk? *Curr Opin Allergy Clin Immunol* 2011; 11:24–28.
 41. Martinez FD. The origins of asthma and chronic obstructive pulmonary disease in early life. *Proc Am Thorac Soc* 2009; 6:272–277.
 42. Ball TM, Castro-Rodriguez JA, Griffith KA, *et al.* Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000; 343:538–543.
 43. Lee KK, Hegele RG, Manfreda J, *et al.* Relationship of early childhood viral exposures to respiratory symptoms, onset of possible asthma and atopy in high risk children: the Canadian Asthma Primary Prevention Study. *Pediatric Pulmonol* 2007; 42:290–297.
 44. Gern JE. The ABCs of rhinoviruses, wheezing, and asthma. *J Virol* 2010; 84:7418–7426.
 45. Message SD, Johnston SL. The immunology of virus infection in asthma. *Eur Respir J* 2001; 18:1013–1025.
 46. Forno E, Onderdonk AB, McCracken J, *et al.* Diversity of the gut microbiota and eczema in early life. *Clin Molec Allergy* 2008; 6:11.
 47. Miller EK, Williams JV, Gebretsadik T, *et al.* Host and viral factors associated with severity of human rhinovirus-associated infant respiratory tract illness. *J Allergy Clin Immunol* 2011; 127:883–891.
 48. Kieninger E, Fuchs O, Latzin P, *et al.* Rhinovirus infections in infancy and early childhood. *Eur Respir J* 2013; 41:443–452.
- Review paper that provides and overview of rhinoviral infections in infancy and early childhood. Reviews the different types of rhinovirus infections and the possible mechanisms that may be involved in disease susceptibility.
49. Jackson DJ, Evans MD, Gangnon RE, *et al.* Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med* 2012; 185:281–285.
 50. Dreyfus DH. Herpesviruses and the microbiome. *J Allergy Clin Immunol* 2013; 132:1278–1286.
- Review paper that evaluates the intersection of herpesviruses and the microbiome.
51. Olenec JP, Kim WK, Lee WM, *et al.* Weekly monitoring of children with asthma for infections and illness during common cold seasons. *J Allergy Clin Immunol* 2010; 125:1001–1006 (e1).

52. Soto-Quiros M, Avila L, Platts-Mills TA, *et al.* High titers of IgE antibody to dust mite allergen and risk for wheezing among asthmatic children infected with rhinovirus. *J Allergy Clin Immunol* 2012; 129:1499–1505 (e5).
53. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. *Clin Microbiol Rev* 2013; 26:135–162.
Comprehensive review of the classification of human rhinoviruses, immune response and host response mechanisms.
54. Camargo CA Jr. Human rhinovirus, wheezing illness, and the primary prevention of childhood asthma. *Am J Respir Crit Care Med* 2013; 188:1281–1282. Editorial review of Cox *et al.* (reference 24) and suggested that wheezing with rhinovirus infection could be used as a 'stress test' to identify children at high risk for the development of asthma and allergic diseases.
55. Adler FR, Kim PS. Models of contrasting strategies of rhinovirus immune manipulation. *J Theor Biol* 2013; 327:1–10.
Mathematical models for viral infection and progression. Model of infection of a highly adapted and low virulence virus provides a starting point for understanding the development of asthma and other respiratory phenotypes.
56. Cadman RV, Lemanske RF Jr, Evans MD, *et al.* Pulmonary 3He magnetic resonance imaging of childhood asthma. *J Allergy Clin Immunol* 2013; 131:369–376 (e1-5).
Investigators used pulmonary ³He MRI to detect abnormalities in lung ventilation and structure in a subset of children from the COAST study who experienced wheezing with viral infection during the first 2 years of life. MRI results demonstrated more and larger regions of ventilation defect and greater degree of restricted gas diffusion in children with asthma compared with those seen in children without asthma, indicating that viral infection and subsequent immune responses may play a role in the airway remodeling that occurs in asthmatic children.
57. Collison A, Siegle JS, Hansbro NG, *et al.* Epigenetic changes associated with disease progression in a mouse model of childhood allergic asthma. *Dis Model Mech* 2013; 6:993–1000.
Results from a mouse model of asthma indicate that progression to an asthmatic phenotype after neonatal infection with pneumovirus, followed by sensitization to ovalbumin is linked to epigenetic regulation of genes associated with inflammation and structural remodeling and with T-cell commitment to a TH2 immunological response. Authors conclude that epigenetic changes associated with this pattern of gene activation may play a role in the development of childhood asthma in humans.
58. Adkins B. Neonatal immunology: responses to pathogenic microorganisms and epigenetics reveal an 'immunodiverse' developmental state. *Immunol Res* 2013; 57:246–257.
Review of numerous neonatal animal models and how evidence from these studies and their observations of responses to mucosal surfaces in early life may be protective against primary and secondary disease. Review of the immunological responses and the properties of neonatal immune cells and maturation of the immune system indicate that these systems are regulated by epigenetic phenomena.
59. Ernberg I, Karimi M, Ekstrom TJ. Epigenetic mechanisms as targets and companions of viral assaults. *Ann N Y Acad Sci* 2011; 1230:E29–E36.
60. Suarez-Alvarez B, Rodriguez RM, Fraga MF, Lopez-Larrea C. DNA methylation: a promising landscape for immune system-related diseases. *Trends Genet* 2012; 28:506–514.
61. Hilty M, Burke C, Pedro H, *et al.* Disordered microbial communities in asthmatic airways. *PLoS One* 2010; 5:e8578.
62. Ly NP, Litonjua A, Gold DR, Celedon JC. Gut microbiota, probiotics, and vitamin D: interrelated exposures influencing allergy, asthma, and obesity? *J Allergy Clin Immunol* 2011; 127:1087–1094.
63. Elazab N, Mendy A, Gasana J, *et al.* Probiotic administration in early life, atopy, and asthma: a meta-analysis of clinical trials. *Pediatrics* 2013; 132:e666–e676.
Meta-analysis of 25 studies that evaluated the effectiveness of probiotic administration in early life and the subsequent development of atopy and asthma. Authors found that probiotics reduced the risk of atopic sensitization (both skin prick test and elevated specific IgE's). However, *Lactobacillus acidophilus* as compared with other strains was associated with an increased risk for atopy. No effect was observed for asthma.
64. Bisgaard H, Li N, Bonnelykke K, *et al.* Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol* 2011; 128:646–652 (e1-5).
65. Abrahamsson TR, Jakobsson HE, Andersson AF, *et al.* Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy* 2014; 44:842–850.
Childhood birth cohort from Sweden recruited children from 2001 to 2005 to evaluate the efficacy of probiotic supplementation on the primary prevention of asthma and allergic diseases. Study included 20 children with IgE-mediated eczema and 27 nonatopic children. Stool samples were collected at 1 week, 1 month, and 1 year of life. Investigators found associations between the diversity of the microbiome at 1 week and 1 month of life and subsequent asthma at 7 years. No association was found at 1 year of life suggesting that effects happen early in development. Study is the first to evaluate/demonstrate an association with asthma but this is likely due to the elongated follow-up time necessary (7 years) to evaluate asthma associations. Findings need confirmation but this will likely be coming soon as there are other birth cohorts (such as the Canadian CHILD birth cohort) that are performing similar studies but do not yet have the necessary follow-up time to evaluate associations with asthma.
66. Ismail IH, Oppedisano F, Joseph SJ, *et al.* Reduced gut microbial diversity in early life is associated with later development of eczema but not atopy in high-risk infants. *Pediatric Allergy Immunol* 2012; 23:674–681.
67. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy* 2000; 30:194–200.
68. Salam MT, Li YF, Langholz B, Gilliland FD. Early-life environmental risk factors for asthma: findings from the Children's Health Study. *Environ Health Perspect* 2004; 112:760–765.
69. Ege MJ, Strachan DP, Cookson WO, *et al.* Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol* 2011; 127:138–144.
70. Gosalbes MJ, Llop S, Valles Y, *et al.* Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. *Clin Exp Allergy* 2013; 43:198–211.
One of the few studies that has investigated infant meconium (fetal fecal samples). Investigators found that meconium samples clustered into two types with different bacterial diversity and composition. One of the types was less diverse, mainly comprised enteric bacteria and was associated with a history of maternal atopic eczema. The second type was dominated by lactic acid bacteria and was associated with respiratory problems in the infant.
71. Azad MB, Konya T, Maughan H, *et al.* Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. *Allergy Asthma Clin Immunol* 2013; 9:15.
Results from investigation of the intestinal microbiome in infants from the Canadian Healthy Infant Longitudinal Development Study (CHILD) demonstrate that there are differences in both the types and abundance of bacteria in infant households with pets and older siblings. Both birth order and dog ownership are protective for the development of asthma and allergic diseases. Results suggest that modification of the infant gut microbiome may be a possible mechanism for these protective effects.
72. Bernsen RM, de Jongste JC, van der Wouden JC. Birth order and sibship size as independent risk factors for asthma, allergy, and eczema. *Pediatr Allergy Immunol* 2003; 14:464–469.
73. Chan-Yeung M, Hegele RG, Dimich-Ward H, *et al.* Early environmental determinants of asthma risk in a high-risk birth cohort. *Pediatr Allergy Immunol* 2008; 19:482–489.
74. Goleva E, Jackson LP, Harris JK, *et al.* The effects of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med* 2013; 188:1193–1201.
One of the few studies to investigate the airway microbiome. The role of the airway microbiome in response to corticosteroids in asthmatic patients was investigated in 39 patients with asthma 29 of whom were resistant to corticosteroids (CR) and 10 responsive individuals (75). Researchers found that the microbiome did not differ in diversity or composition at the phylum level.