# Chapter 5
# Asthma and Microbes: A New Paradigm

## 5.1 Introduction

Asthma is a heterogeneous disorder with interaction between genetic predisposition, atopy, and environmental factors, including environmental allergens, air pollution, and respiratory infections. The current definition of asthma combines clinical–pathological presence of chronic inflammatory airway with hyperresponsiveness and episodic airway obstruction with variable degrees of reversibility with treatment or spontaneously [1]. Asthma is worldwide and is of pandemic levels for the past 30 years, with >300 million people afflicted globally [2]. Although asthma is considered more common in affluent and developed countries, approximately half are in developing countries [3], which account for more than two-thirds of the world's population. It is estimated that asthma accounts for 25,000 annual deaths, and that by 2025 the number of asthmatics will increase by more than 100 million new cases [2, 4]. In the United States 8.4 % of the population has asthma with substantial annual morbidity of 500,000 hospitalization and 1.9 million emergency visits, at an annual cost of $56 billion [5].

## 5.2 Pathogenesis of Asthma

Traditionally asthma has been considered a condition as a result of a complex interaction between multiple genetic influences and environmental stimuli. Studies of twins in families of subjects with asthma show an inheritable pattern but the current data indicate that asthma is likely transmitted by multiple genes [6]. Different genes may lead to the same phenotype in separate individuals [locus heterogeneity], and multiple genes acting in the same individual [polygenic inheritance] may lead in the expression of the asthma phenotype [6]. Some genes influence the development of asthma and others may influence the severity of the disease or responses to treatment. Genome-wide association studies have recently identified the number of genes that are



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important in the development of asthma. Two key genes, IL-33 and IL-1 receptor-like 1 [IL1RL1], act in this signal transduction pathway [7]. IL-33 encodes a cytokine released on damages cells and IL1RL1 encodes part of the receptor complex. Functional studies in humans and mouse models of allergic airway disease indicate a key role of IL-33 signaling in driving the Th2 inflammation, which is pivotal in allergic asthma [7]. The IL-33/IL1RL1 pathway can activate innate immune cells to produce cytokines such as IL-5 and IL-13 in the lungs [8, 19].

In a previous review of asthma genetics in 2006 [10], there were 118 genes implicated or associated with asthma or atopy related phenotypes, 25 genes were considered true susceptibility genes, but there were 10 elite groups of genes associated with asthma or atopy in more than 10 studies. This elite group of genes, include those encoding IL-4, IL-13, ADRB2, TNF, HLA–DRB, FCER1B, IL4RA, CD14, HLA-DQB1, and ADAM 33 [10]. Asthma susceptibility genes may be classified according to four main functions: Innate immunity and immunoregulation [e.g., CD14, HLA genes, and TLR4]; Th2-cell differentiation/activity [e.g., IL4/IL4R, IL 13, and FCER1B]; epithelial biology and mucosal immunity [e.g., CCL genes and FLG]; and lung function, airway remodeling, and asthma severity [e.g., ADRB2 and TNF] [11, 12]. IL-13 has been one of the best studied candidate gene for asthma and allergy, and has been consistently implicated in genome-wide based studies [13, 14].

The pathogenesis of asthma may also involve epigenetics, chemical reactions that may switch parts of the genome on and off, and thus may be one of the mechanisms for interaction of the genome with the environment [6]. For example, environmental factors may cause changes in gene expression through noncoding changes to the DNA, i.e., DNA methylation [15]. There are several observations supporting a pathogenic role of epigenetics, including the complexity of gene–environment interactions. The concordance rate of only 50 % in monozygotic asthmatic twins supports nongenetic factors [16]. Common epigenetic mechanisms, such as prenatal exposure to tobacco smoke or air pollutants altering DNA methylation, may result in increased or decreased gene expressions [121]. For example, expression of ADAM 33 in bronchial epithelial and fibroblasts cells has been shown to be controlled by epigenetic mechanisms [12]. Currently epigenetic analyses in respiratory diseases have been limited to certain candidate genes, i.e., FOXP3 involved in regulation of T-cell function and no large-scale studies have been published [17].

### *5.2.1 Pathological Aspects of Asthma*

The hallmark of asthma from a pathological perspective is the presence of inflammation with smooth muscle contraction and largely reversible airway obstruction. The inflammation and exaggerated airway responsiveness is associated with mucus hypersecretion, often with increased eosinophils locally and systemically. At the cellular level the changes consist of airway remodeling characterized by smooth muscle hyperplasia, subepithelial cell fibrosis, goblet cell hyperplasia, and neovascularization [18]. The airway inflammation which is a major component of

asthma involves influx of mast cells, neutrophils, Th-2 cells, and eosinophils. On exposure to allergen or infectious agent the physiological changes that occur are driven by Th-2 inflammation, with increased Th-2 cytokines [IL-4, IL-5, IL-9, and IL-13], and decreased anti-inflammatory cytokines, i.e., IL-10 [18]. The heightened Th-2 response causes activation of natural killer cells, dendritic cells and eosinophils, which result in eosinophilia, increased matrix metalloprotease activity, increase in serum IgE levels, and promotion of smooth muscle hypercontraction. IL-13 is central to the progression of asthma and is produced in response to toll-like receptor-4 [TLR-4] signaling, and many micro-RNAs are directly or indirectly involved in the production and upregulation [18].

Asthma may be classified as two forms, based on history and skin test reactivity to common inhaled allergens, atopic asthma in over 80 % of cases and nonatopic or intrinsic asthma in about 10 % [19]. Nonatopic asthma is usually late or adult onset with normal serum IgE levels, and more commonly associated with nasal polyps and aspirin sensitivity. However, there is data to suggest that IgE-mediated mechanism in the airway is involved and staphylococcal enterotoxins have been implicated [19]. The nonatopic form of asthma may be the commonest form in children of developing countries and has been related to exposures to environmental dirt, bacterial infection, and psychosocial distress of poverty [20].

## 5.3 Infection and Asthma

Microbes have been recognized to be important in the exacerbation of asthma with precipitations of severe attacks for many decades. The pathogenic mechanisms could be either from respiratory infections worsening bronchial inflammation or allergic reaction to environmental molds as in allergic bronchopulmonary aspergillosis. However, in recent years there has been cumulative evidence that repeated lower respiratory viral infections in early childhood may be responsible for later development of asthma. Conversely, lack of environmental exposures to common microbes in early life may be responsible for the asthma epidemic in developed countries, as part of the "hygiene hypothesis" of allergic diseases.

### *5.3.1 Asthma Exacerbations and Infection*

Respiratory viruses account for 50–60 % of asthma exacerbations in all age groups [21, 22]. Rhinoviruses [RV], common cause of the "common cold," are the most frequent culprit and the genotypes C, [RV–C] may cause more severe exacerbation than other respiratory viruses [23]. Although RV–C infections are associated with asthma, recurrent wheezing, and bronchiolitis in children admitted to hospital with respiratory tract infection, no clinical differences were found from the RV–A genotype in one study [24]. In temperate regions infections with RV or other respiratory

viruses are associated with increased emergency room admissions for asthma exacerbations, and "asthma epidemics" have been associated with children returning to school in September [21]. In animal models RVs have been shown to exacerbate the airway inflammation induced by airway allergen challenge [25], and this is likely similar for other respiratory viruses as well.

Bacterial infections were until recently not considered important in the pathogenesis of asthma. However, there is evidence that asthmatics have increased susceptibility to bacterial respiratory infections. In children pneumococcal carriage is more common in asthmatics than nonasthmatics, and adult asthmatics have increased risk of invasive pneumococcal infections [26, 27]. There is also increasing evidence that *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* may play a role in promoting airway inflammation that could contribute to the onset and course of asthma [28]. Neonates colonized in the hypopharynx with *Streptococcus pneumoniae* and *Haemophilus influenzae* and *Moraxella catarrhalis* are at increased risk for recurrent wheeze and asthma in early life [29].

Prospective studies in Europe have also found increased frequency of *C. pneumoniae* detection by RT-PCR from asthmatics compared to their normal spouses from October to December, 22 % versus 9 % [30]. Bronchial lavages of children with asthma were also found to have slightly greater prevalence of *C. pneumoniae* [40 % by PCR and 20 % by cultures] compared to nonasthmatics with various respiratory disorders [35.7 %]. However, blood culture positivity for *C. pneumoniae* from asthmatics [40.5 %], other respiratory disorders [29 %] versus matched nonrespiratory controls [11 %] was significantly greater, $p < 0.01$ [31].

The role of viruses and atypical bacteria in exacerbation of asthma for hospitalized children was prospectively evaluated in Europe [France] over a 9-month period in 15 hospitals. Viruses were detected in 38 % [enterovirus 15.8 %, RV 12 %, and respiratory syncytial virus [RSV] in 7 %], and atypical bacteria in 10 % [32]. Persistent clinical symptoms were more frequently associated with atypical bacterial infections. A similar prospective study was performed in the Southern hemisphere [Argentina] over a year [33]. Two hundred nine patients were assessed and a potential causative agent was detected in 78 % of the children. The most frequently detected viruses were RSV [40 %] and RV [24.5 %], with *M. pneumoniae* and *C. pneumoniae* in only 4.5 % and 2 % of the cases, respectively [33]. The impact of acute respiratory infections with viruses and atypical bacteria, on severity and resolution of symptoms, in children with nonhospitalized exacerbation of asthma was also assessed in Australia [34]. Respiratory viruses were detected in 54 % of 78 nasopharyngeal aspirates but there was no difference in clinical outcome between those with or without proven infection.

Currently the overall data from multiples studies indicate that viruses are much more important and frequent than atypical bacteria in precipitating exacerbations of asthma. There is no good evidence that this is related to any specific respiratory virus. The relative frequency of the various respiratory viruses reported may vary according to age group, season, and geography, as well as methods of detection. A large diversity of viruses have been found in the respiratory tracts of adults with and without asthma using the Virochip, a DNA microarray-based

detection method [35]. These include a set of 5 divergent isolates that formed a distinct genetic subgroup, besides >20 different serotypes of RVs and multiple serotypes of human coronaviruses.

The exact mechanism by which upper respiratory tract viral infection induces asthma attacks is not fully understood. In established asthma respiratory viral infections attract bronchial inflammatory cells, alter airway receptor expression on smooth muscle cells, and modulate neuroimmune mechanisms, leading to bronchospasm and exacerbation of disease [36]. Multiple mechanisms are probably involved including loss of function or downregulation of M2 muscarinic receptors on the airway parasympathetic nerves, which normally inhibit vagal-mediated bronchospasm [37]. Interferons produced in response to viral infections downregulate the expression of the M2 receptor gene, and eosinophils recruited by allergens release major basic proteins, which bind to the M2 receptors and block their function [37]. Thus a multitude of respiratory viruses that can cause upper and lower respiratory tract infections and a few atypical bacteria can cause exacerbation of asthma.

### *5.3.2 Viruses in Early Life as a Cause of Asthma*

Lower respiratory viral infections commonly cause wheezing in young children <2 years of age, and there is increasing evidence that repeated viral bronchiolitis in early life predispose to asthma in later childhood. Development of new onset of asthma has been correlated with a variety of respiratory viruses, most commonly RSV bronchiolitis in infancy and RV infection in older children [39]. Wheezing illness in infancy is most commonly caused by marked RSV bronchiolitis [70 % of episodes], and less frequently with parainfluenza virus, influenza, and metapneumoviruses [39]. Although RSV infection is almost universal in young children only about 40 % develop clinically overt disease and some develop severe bronchiolitis with recurrent infection and wheezing. Risk factors for severe lower respiratory tract infection include younger age, small lungs size, passive smoke exposure, and virus-induced immune responses [39].

In the Tucson Children's Respiratory Study 880 children were followed from birth for over a decade, and development of lower respiratory tract infection in the first 3 years of life was later correlated with diagnoses of asthma or recurrent wheezing at ages 6 and 11 years [40]. Although RSV bronchiolitis increase the risk of frequent [>3 episodes per year] or infrequent [<3 episodes per year of wheezing], the risk gradually decreased by age 13. The results of this study may be explained by a more recent study assessing recurrent wheezing and development of asthma in a birth cohort followed for 13 years, and the relationship to perennial allergen exposure and sensitization [41]. Chronic asthma developed in atopic children in the first 3 years of life to repeated exposures to allergens, and determined by continuing allergic airway inflammation characterized by airway-hyperresponsiveness and impairment of lung function at a later age school age. Whereas children with nonatopic wheezing phenotype lose their symptoms over school age and retain normal

pulmonary function at a later age [41]. Other studies have suggested that RSV bronchiolitis predispose to recurrent wheezing and asthma in the genetically predisposed children [42, 43]. A large epidemiological study from Tennessee [USA] found that children born approximately 120 days before the peak RSV season were at the highest risk for hospital admission with wheezing, and at a higher risk for later development of asthma [44]. However, the association of severe RSV infection in infancy and later development of asthma overlapped with genetic determinants in a study of 8,280 twin-pairs [45]. The investigators of this study concluded that severe RSV infection may not be the cause of asthma, but there was a genetic predisposition to both RSV bronchiolitis and asthma. The best evidence to date in support of a causal association between RSV infection and asthma is the result of the long-term study over 18 years in Swedish children. Children who had RSV bronchiolitis as infants compared to matched controls had a greater prevalence of asthma at 18 years old, 39 % versus 9 % [46]. The affected children also demonstrated increase in perennial allergens sensitization [41 % versus 14 %] and recurrent episodes of wheezing, 30 % versus 1 %. In a previous study children with past bronchiolitis had an enhanced IL-4 response to RSV and cat allergen [Th-2 response], whereas controls had an equally strong interferon-gamma [IFN-γ] response [Th-1 type] to RSV antigens [47].

Controlled studies with prophylactic therapy with anti-RSV monoclonal antibody [palivizumab] in infancy also support a causal role of RSV in asthma. In one study of 191-palivizumab treated and 230 untreated premature babies, the rate of recurrent wheeze was 50 % lower at 24 months in the treated babies [48]. The investigators later showed that RSV prophylaxis treatment had a similar protection against recurrent wheeze in older children age 2–5 years. However, the protective effect of recurrent wheezing was primarily in children without a family history of asthma or atopy, and no effect on subsequent wheezing was demonstrated in 90 children with the family history of asthma or atopy [49].

Human rhinoviruses [RVs] are recognized as a common cause of the "common cold" and exacerbation of asthma, but these viruses can also cause lower respiratory tract disease leading to hospitalization in children with or without asthma [50]. There is increasing evidence that RV infections can lead to the development of asthma [50], and bronchial epithelial cells of asthmatics have a deficient innate immune response to RV infection [51]. In a recent prospective longitudinal study of 285 children with high risk for allergic diseases and asthma followed from birth, allergic sensitization leads to increased risk of RV-induced wheezing but not RSV infection up to 6 years [52]. However, viral wheeze did not lead to subsequent allergic sensitization. In an earlier study sensitization to common aeroallergens nearly doubled the risk for asthma at 6 years, and viral respiratory tract infection with wheezing quadrupled the risk for asthma over this time [53]. The combined effect appeared to be synergistic as the risk of asthma was ninefold greater with both wheezing episodes from respiratory infections and allergic sensitization. Recent evidence also suggests that RV infection induces bronchial epithelial production of a number of growth factors and other mediators [chemokines and cytokines] that could contribute to the development and progression of airway remodeling present in asthma [54].

### 5.3.3 *Mechanisms of Virus-Related Asthma*

A variety of mechanisms have been proposed to explain increased susceptibility of asthmatics for certain respiratory pathogens. These include impaired innate immunity in asthma and atopic diseases, including deficient epithelial cell function, mucus hypersecretion, decrease in interferon responses, and impaired alveolar macrophage function [55]. However, it is unclear how repeated viral bronchiolitis in early childhood could lead to asthma in later years.

Viral bronchitis or bronchiolitis precipitation of acute attacks of wheezing or exacerbation of asthma seems to involve local inflammation of the epithelial lining and enhanced airway bronchoconstriction. Experimentally induced viral infections in animals and human volunteers indicate that viruses can enhance airway hyperresponsiveness [56]. Virus-induced airway hyperresponsiveness is multifactorial and involves changes in neural control of the airways, impaired inactivation of tachykinins, and effects on nitric oxide production [57]. There is further evidence that atopic or allergic subjects with increased IgE and eosinophilic airway inflammation have greater risk of virus-induced wheezing than nonallergic individuals [58]. Although several studies suggest that there is an interaction between response to viral infection and aeroallergens in asthma the mechanisms are unclear. Viral infections could damage the epithelial airway barrier to enhance absorption of aeroallergens and thus enhance airway inflammation [59]. Generation of various cytokines and chemokines by viral infection may upregulate cellular recruitment to enhance allergen-induced inflammation and airway hyperresponsiveness. Experimental RV infection can enhance lower airway histamine responses and eosinophils recruitment to allergen challenge [60]. The possibility that allergic inflammation might intensify the host response to viral respiratory infections or impair viral clearance has been suggested by some studies but not confirmed by others [39].

The evidence that the strongest predictor of subsequent asthma is the presence of both atopy and severe lower viral respiratory tract infections in infancy suggests that there is a virus–allergen interaction at least in some asthmatics. This simply could imply that there is a common predisposition or susceptibility to both asthma and viral infections [61]. It has been hypothesized that severe, recurrent lower viral expiratory infections hinder proper lung growth and development at a very vulnerable age, leading to changes in the airway structure that promote asthma [62]. There is experimental evidence that RSV and RV in vivo and in vitro increase the synthesis of factors that can modulate lung growth, development, and repair [63, 64].

A central role in the pathogenesis of asthma is the allergen-specific IgE, the production of which is mediated by the Th-2 cytokines, IL-4 and IL-13 [38]. IgE receptors on mast cells and basophils bind to allergens, resulting in release of mediators such as histamine which produce airway inflammation and hyperreactivity. It has been proposed that the immune dysfunction in infancy could predispose to recurrent viral lower respiratory tract infection and be involved in the pathogenesis of asthma. In a community-based cohort of children, with a family history of atopy, relative deficiency of circulating plasmacytoid dendritic cells in infancy was correlated

with increased frequency and severity of viral respiratory infections, wheezing, and diagnoses of asthma [65]. The usual response to viral respiratory infections by a mature immune system is to stimulate proliferation of cytolytic T cells and the Th1-CD4 helper T cells. This results in release of IFN-γ, upregulation of IL-12, and stimulation of macrophages [66], typical for the Th1 response. However, there is experimental evidence that severe RSV infection in neonates could determine the pattern of T-cell-mediated disease during later life. Primary RSV infection in neonatal mice was associated with reduced and delayed IFN-γ responses, and subsequent reinfection resulted in increased inflammatory cell recruitment, with a shift to increased Th2-cytokine and increased eosinophils, typical for allergy and asthma [67]. A similar animal model also confirmed that viral respiratory infection in early life results in overproduction of the Th2-cytokines, IL-13, with increased risk for respiratory allergies, and changes in the airway structure conducive for asthma [68]. In clinical studies of acute exacerbation of asthma induced by viral infection, the peripheral blood mononuclear cells demonstrate increased expression of IFN-responsive genes and increased expression of Th2-chemokines, indicating enhanced allergic inflammation linked to the innate antiviral response [69, 70].

## 5.4 The Hygiene Hypothesis of Asthma

In 1989 Strahan first observed that the risk of allergic rhinitis [hay fever] was inversely linked to the size of the family and hygiene [71]. He proposed that increased infection in childhood had a protective role in allergic rhinitis and this concept was labeled the "hygiene hypothesis". However, it has since been noted by others that repeated viral lower respiratory tract infections in the first 3 years of life appear to be associated with asthma at 7 years of age, whereas recurrent upper respiratory tract infection in early life reduced the risk of asthma at school age [72]. Multiple epidemiological studies from Europe and elsewhere have shown that people living on farms from childbirth through to adults, especially with exposure to livestock or consumption of unpasteurized milk, have significantly lower prevalence of asthma and allergic rhinitis than the general population [73]. This is presumably due to greater environmental exposure to higher burden of multiple pathogens and microbes. The timing and duration of exposure to unhygienic environment appears to be critical, as the reduction in risk of allergic disease is greatest for those exposed prenatally and continuously until adulthood [73]. The maternal exposure to animal sheds and unpasteurized cow's milk has also been shown to influence the production of IgE antibodies in the cord blood of neonates [74]. Furthermore, children living on farms are exposed to higher bacterial components, endotoxin and muramic acid, from abundant mattress dust compared to nonfarm children. Exposure to the microbial laden mattress dust has been associated with lower frequency of wheezing, asthma, and hay fever [75–77]. Similar findings were reported in a case-controlled study from Chile, where daycare attendance and regular farm animal contact were inversely related to childhood asthma [78]. In a case-controlled study from Brazil the

effect of crowding in the home environment was also associated with the development of asthma, crowding in the home was associated with a 60 % reduction in incidence of asthma, yet there was a 2.5-fold increase in incidence of lower respiratory tract infections [79].

The microbial burden and exposures in homes, even in Urban areas, may vary with overcrowding, presence of pets, and social status. In a longitudinal birth cohort study from Boston with enrollment of approximately 500 children, followed until school age, dust samples were collected and analyzed for bacterial biomarkers [80]. Multiple exposures to both gram-negative and gram-positive bacteria were associated with decreased asthma, and school age, endotoxin exposure remained protective for allergic disease adjusted for early life endotoxin. In a preliminary study utilizing molecular methods [PCR–the nature ring gradient gel analysis] to analyze home dust, differences in the dust bacterial community were associated with asthma outcomes in young children, including wheezing and specific IgE [81]. The bacterial community structure of the house dust was significantly impacted by the presence of dogs or cats in the homes. Experimental findings in pregnant mice with asthmatic phenotype indicate that prenatal exposure with farm-derived bacteria operate by means of epigenetic mechanisms, altering the activity of genes without changing their structure to protect against transmaternal asthma [82].

Not all studies, however, support the hygiene hypothesis in the pathogenesis of asthma. In a longitudinal study of birth cohort, 3,963 newborn children were prospectively followed for 8 years in the Netherlands [83]. Early daycare provided no protection against asthma or allergic sensitization at the age of 8 years. A prospective study of allergic prone children from birth to 6 years [$n = 620$] was assessed for the effect of early childhood infections and immunizations on the development of asthma in Melbourne, Australia [84]. Recurrent gastroenteritis in early childhood was associated with greater risk of asthma, and Sabin polio vaccination in the second year of life was associated with a decreased risk.

In Africa studies have shown that allergic diseases have shown a steady increase over the past 10 years or more. Allergic diseases, IgE, and skin reactivity to allergens increase with increasing affluence and greater gross national income of the countries [85]. Association between helminthic infections and allergies was contradictory but rural living was associated with a decreased risk of allergic diseases [85].

### *5.4.1 Microbial Colonization and Asthma*

In a cross-sectional study of 7,412 children [3–19 years of age], participants of the National Health and Examination Survey [NHANES], *Helicobacter pylori* seropositivity was inversely associated with asthma in children [86]. Colonization of the gastrointestinal tract may be a reflection of sanitation and exposure burden of microbes and this could support the hygiene hypothesis. The same investigators had previously reported that colonization with *H. pylori* [Cag A+] in adults was inversely

associated with the presence of asthma or allergic rhinitis in a study of 7,663 subjects [87]. The combined infectious burden of *H. pylori*, *Toxoplasmosis gondii*, hepatitis A, herpes simplex 1, *C. pneumoniae*, Epstein–Barr virus, and *Cytomegalovirus* [determined by presence of antibodies] was associated with lower risk of atopy, asthma, and allergic rhinitis in 1,249 adults in Europe [88]. However, in other cross-sectional studies in adults there was no association of *H. pylori* serological status and asthma or atopy [89]. Although in an experimental mouse model of allergic asthma, *H. pylori* infection could prevent asthma development through the induction of regulatory T cells [90].

The relationship between intrauterine bacterial colonization at delivery and development of asthma 15–17 years later in 460 children has been reported from Finland [91]. In vitro growth of pathogenic anaerobic bacteria, and streptococcus species from the maternal womb at birth was associated with significant increased risk of asthma diagnosis compared to those with negative bacterial cultures. There are several limitations of this study, however, including the small sample size and failure to provide information on other potential risk factors, such as household environments and previous lower respiratory tract infection in infancy. This study may be worthwhile repeating in a larger population with molecular methods to assess the microbial environment of the vagina, uterine cavity, and the household dust, especially in women with a family history of asthma or atopic diseases.

Can oral microbial pathogens influence development of allergic diseases? This topic was reviewed in 2011, with the conclusion that it is biologically plausible that oral bacteria through immune mechanisms could influence the risk of allergic diseases, but the data was insufficient to draw any conclusions [92]. In a childhood birth cohort prospective study from Copenhagen, aspirates of the hypopharynx were obtained from asymptomatic 1-month-old infants of asthmatic mothers for bacterial culture and subsequently correlated with later development of asthma at 5 years of age [29]. Colonization of the hypopharynx with *S. pneumoniae*, *H. influenzae*, or *Moraxella catarrhalis* or with a combination of these organisms was at increased risk for recurrent wheeze and asthma in early life. A subsequent study has shown that polymorphism of the IL-17 gene was associated with childhood asthma and bacterial colonization of the hypopharynx in bronchiolitis [93]. Thus bacterial colonization of the hypopharynx in neonates and childhood asthma may be linked to a genetic predisposition. However, there is no evidence that antibiotics use in early life decreases or increases the risk for later development of asthma [94]. It has been postulated that bacterial colonization of the airway could be related to development of IgE against bacterial antigen, implicating a role for bacterial-specific type-2 immunity in the pathogenesis of asthma. Titers of IgE against *H. influenzae* and *S. pneumoniae* and *Staphylococcus aureus* were measured in 1,380 teenagers and correlated with asthma and immunophenotypes [95]. IgE titers against *S. aureus-derived* enterotoxins were highest among atopic subjects and were associated with increased risk of asthma. However, high IgE titers against *H. influenzae* and *S. pneumoniae* were associated with a decreased risk of asthma. The investigators postulated that lower availability of soluble forms of *H. influenzae* and *S. pneumoniae* antigens reduces the cross-link with IgE receptors systemically, but the availability of

these antigens at the mucosal level to antigen presenting cells and type-2 memory cells could lead to mucosal secretion of IL-4/IL-13 producing an atopic response [95].

*S. aureus* enterotoxin [a super-antigen]-specific IgE antibodies have been associated with asthma severity and the various phenotypes in other studies [96]. In a review and meta-analysis of ten studies, patients with asthma or allergic rhinitis were more likely than controls to have serum-specific IgE to *S. aureus* enterotoxins [97]. In a more recent case-controlled study *S. aureus* enterotoxin IgE antibodies, but not IgE against inhalant allergens [grass pollen and house dust mite], were risk factors for asthma severity [98].

The intestinal microbiota appears to be important in development of the host innate immunity and may play a role in the pathogenesis of other allergic and autoimmune diseases. A recent study investigated the relationship between fecal microbiota composition, mode, and place of delivery with atopic diseases [99]. Fecal samples were collected from neonates at age 1 month [$n = 1{,}176$] to determine microbiota composition, and blood samples were collected at ages 1, 2, and 6–7 years to determine specific IgE levels. Colonization by *Clostridium difficile* at 1 month of age was associated with wheezing and eczema throughout the first 6–7 years of life and with asthma at age 6–7 years. Vaginal delivery at home was associated with decreased risk of eczema, food allergy, and asthma in comparison to vaginal delivery in hospital [99].

Previous but smaller prospective birth cohort of 117 children had found that *Bacteroides fragilis* fecal colonization at the age 3 weeks was an indicator of possible asthma in later life, asthma predictive index was positive in 64 % versus 34 % in those without *B. fragilis*, $p < 0.05$ [100]. In another study of 76 infants at high risk for atopic diseases, intestinal microbiota were analyzed at 3 weeks and 3 months of age and subsequently correlated with skin reactivity at 12 months [101]. Atopic children had more clostridia and fewer bifidobacteria, reduced ratio of bifidobacteria to clostridia than nonatopic infants, $p = 0.03$. Moreover, experimental studies in mice have shown that alterations of the gut microbiota can play an important role in regulating immune responses in the lungs to inhaled antigens [102]. Antibiotic-induced perturbations of the gut microbiota can produce allergic airway response that is mediated by IL-13 and CD4 T cells [102].

There is also evidence that the normal bronchial tree is not sterile and contains a mean of 2,000 bacterial genomes per square centimeter of surface area [103]. Pathogenic *Proteobacteria* [especially Haemophilus species] were significantly increased in asthmatic children and adults than controls; conversely, *Bacteroidetes*, particularly *Prevotella* species, were more frequent in controls than in asthmatics [103]. It remains unclear from the results of this cross-sectional study whether the asthmatic airway predisposed to specific bacteria or vice versa. A subsequent study in 65 adults with suboptimally controlled asthma and 10 healthy controls assessed the bacterial burden of bronchial epithelium by 16S ribosomal RNA amplicon concentration [104]. Bacterial diversity and concentrations were significantly higher among asthmatic patients than controls. The relative abundance of particular phenotypes including members of *Comamonadaceae*, *Sphingomonadaceae*, *Oxalobacteraceae*, and other bacterial families were highly correlated with the

degree of bronchial hyperresponsiveness [104]. These two studies suggest that the microbiome of the airways may contribute to the pathogenesis of asthma. However, another recent study using molecular methods found the bacterial communities of the lungs are indistinguishable from the upper airways, but the microbial biomass was 2–4 logs lower in healthy adults. Thus there is no unique lung microbiota or microbiome.

## 5.5 Microbes and Asthma at the Cellular Level

At the cellular and molecular level the mechanisms of viral lower respiratory tract infections and asthma, and bacterial colonization of the airway, or environmental exposure with development of asthma may be different. Eosinophils, the key effector cells of atopic asthma, are considered the potential link between viral infections and asthma [106]. Recruitment of eosinophils in the airway is the main trigger for inflammation and bronchoconstriction on exposure to aeroallergens, and may play a role in the antiviral immunity to eradicate viruses and to decrease invasion of epithelial cells [106]. Neutrophils, which are considered more important in bacterial infection as part of the innate immune response, appear to be important in the pathogenesis of nonatopic intrinsic asthma. It is estimated that about 20 % asthmatic patients have neutrophilic airway inflammation which is associated with bacterial persistence, such as *H. influenzae*, in the airway and steroid resistance [107].

Host factors are likely important in the association of viral infections in infancy proceeding development of asthma. Polymorphism in genes controlling innate immunity, antiviral and Th1 and Th2 immune responses are associated with both asthma and severe respiratory tract infections in early childhood [108]. However, it is unclear whether or not these genetic predispositions are present in only a fraction of children who develop asthma after severe recurrent lower respiratory infections. There is experimental evidence that viral respiratory infection in atopic children may initiate an atopy-dependent cascade that amplifies and sustains airway inflammation initiated by antiviral immunity by utilizing underlying atopic-associated mechanisms [70]. Recent evidence that anti-IgE monthly prophylactic therapy can significantly reduce asthma exacerbations in children, particularly during the common cold season [109], supports a mechanistic interaction of the viral Th1-type response and the allergic Th2-type response which result in increased IgE. This has been attributed to a defective type-1 response to rhinoviruses in atopic asthma, with reduced IFN-γ and shift toward a type-2 phenotype [108].

The relationship between commensal microbiota colonization in early life and later development of asthma may partly be explained by recent experimental findings. Previous investigations have demonstrated that inflammasome activation and downstream cytokines play a role in innate and adaptive antiviral immune defense in vivo [111, 112]. Recent studies in mice demonstrate the importance of gastrointestinal commensal microbiota in regulating immunity, through establishment of Th1, CTL, and IgA responses in the respiratory mucosa following influenza infection [113].

The data indicate that commensal microbiota responsible for conferring an immunogenic environment in the lungs is either gram-positive bacteria of the gut and possible commensal of the nasal mucosa. These results provide a link between commensal microbiota and inflammasome-dependent cytokine activation [113].

## 5.6 Alternative Hypotheses Linking Microbes and Asthma

Although the hygiene hypothesis is currently in vogue to explain the rising incidence of asthma in prosperous countries, there are other theories. It has recently been postulated that changes in diet, which is different in developed countries from developing nations, and associated changes in the gut microbiota are driving the increasing incidence of autoimmune and allergic diseases in developed countries [114]. Diet itself has considerable effect on the composition of the gut microbiota, and experiments in mice show changes in their microbial composition, metabolic pathways, and gene expression just after 1 day on the Western diet [115]. The Western diet causes an increase in bacteria of the *Firmicutes* phylum and a decrease in those of the *Bacteroidetes* phylum. The gut microbiota of children in Africa is greatly different from those in Europe, and this is attributed primarily to dietary differences [115]. The examples provided by the authors to support the hypothesis of diet rather than hygiene affecting the incidence of asthma are the relatively low prevalence of asthma in Japan compared to Australia and the United States [115]. Japan has high a degree of sanitation and urbanization but much different diet that would influence the gut microbiota/microbiome. In addition, the urban poor in the USA with greater frequency of infection, crowding, and likely less sanitary habits still have a high incidence of asthma.

Vitamin D deficiency could be the common factor linking asthma, allergy, and respiratory infections [116]. Vitamin D is important for effective function of the innate and adaptive immunity. Vitamin D is associated with a dose-dependent reduction in the transcription of Th1-cytokines such as IL-2 and IFN-γ and increase in the Th2-cytokines, IL-4, IL-5, and IL-10, in peripheral blood mononuclear cells culture [116]. Thus vitamin D has a key role in the Th1-Th2 balance. Deficiency of vitamin D is common in children and adults of temperate regions of the world, and is associated with higher risk of upper and lower respiratory infections, especially in children. Only 10 % of vitamin D is acquired from ingested food and 90 % from synthesis after sunlight exposure. Therefore, the higher prevalence of vitamin D deficiency seen in countries of the Northern hemisphere could explain the greater incidence of asthma in prosperous countries compared to poorer nations, which are predominantly located in tropical and subtropical regions of the globe. The link between vitamin D deficiency and respiratory infections is particularly relevant in children who develop asthma after recurrent respiratory tract infection in early childhood. This seasonality of influenza and RSV-induced bronchiolitis has been linked to the greater prevalence of vitamin D deficiency in the winter [117, 118]. In a recent prospective birth cohort study of 156 healthy neonates, low concentration

of vitamin D from cord blood was associated with increased risk of RSV bronchiolitis in the first year of life [119].

Several epidemiological studies have suggested that vitamin D deficiency is associated with increased risk of asthma and allergic diseases [120–124]. In a study from Costa Rica, where sunlight exposure should be uniform throughout the year, insufficient levels of vitamin D were associated with higher eosinophil count and IgE levels and increased airway hyperresponsiveness [124], while higher vitamin D levels were associated with lower risk of asthma exacerbation and hospitalization. These results were confirmed in another study by the same group of investigators in a cohort of 1,024 children, and it was suggested that vitamin D may protect against respiratory infections and symptoms of asthma by reducing bronchial inflammation [125]. This postulate was confirmed by a small double-blind randomized study that showed vitamin D supplementation reduced the risk of asthma exacerbation by respiratory tract infections from fall to spring [126].

However, not all studies have found an association between vitamin D deficiency and asthma and atopy. In fact, some reports suggest that vitamin D supplementation can increase the risk of asthma and atopic diseases [127]. A birth cohort study from Finland found that subjects given regular vitamin D supplements in the first year of life had a somewhat higher risk of asthma, atopy, and allergic rhinitis as adults compared to controls, not previously given supplements [128]. Similarly, a Swedish study reported that high vitamin D intake in infants correlated with greater risk of eczema at 6 years of age [129].

## 5.7 Probiotics for Allergic Diseases

The concept that commensal microbiota of the gut plays an important role in the pathogenesis of asthma and allergic diseases suggests that probiotics should be useful. Atopic eczema is the earliest manifestation of allergic diseases in children, and often precedes development of atopic asthma. In mice feeding of *Lactobacillus casei* strain Shirota was effective in inhibiting IgE production to a commonly used allergen [130]. The value of probiotics in allergic diseases has been assessed in several randomized, controlled trials, but mainly involving subjects with atopic eczema, allergic dermatitis, and allergic rhinitis. The main limitations of these trials have included small sample sizes, heterogeneity, and lack of a standardized acceptable probiotic mixture. This topic was last reviewed by Yao et al. who came to the conclusion that there was insufficient evidence to recommend probiotics for allergic diseases, including asthma [131]. The authors noted that the preventative studies failed to show a significant reduction in atopic sensitization, and there have been reports of probiotics administered during the perinatal period being associated with greater risk of later development of wheezing or asthma [131].

## 5.8 Conclusion

It is generally accepted that viral and some bacterial respiratory tract infections can exacerbate asthma. However, it is still not clear or well established that microbes can cause asthma, but it is biologically plausible that microbes could play a significant role in the pathogenesis of asthma and there is accumulating supportive evidence over the past 10 years. A diagrammatic paradigm of the role of microbes in the pathogenesis of asthma is shown in Fig. 5.1. It is quite possible that microbes influence the development of asthma in different ways, depending on the underlying genetic predisposition and that asthma is truly a heterogeneous group of disorders with different pathogenic mechanisms but with similar clinical manifestations.

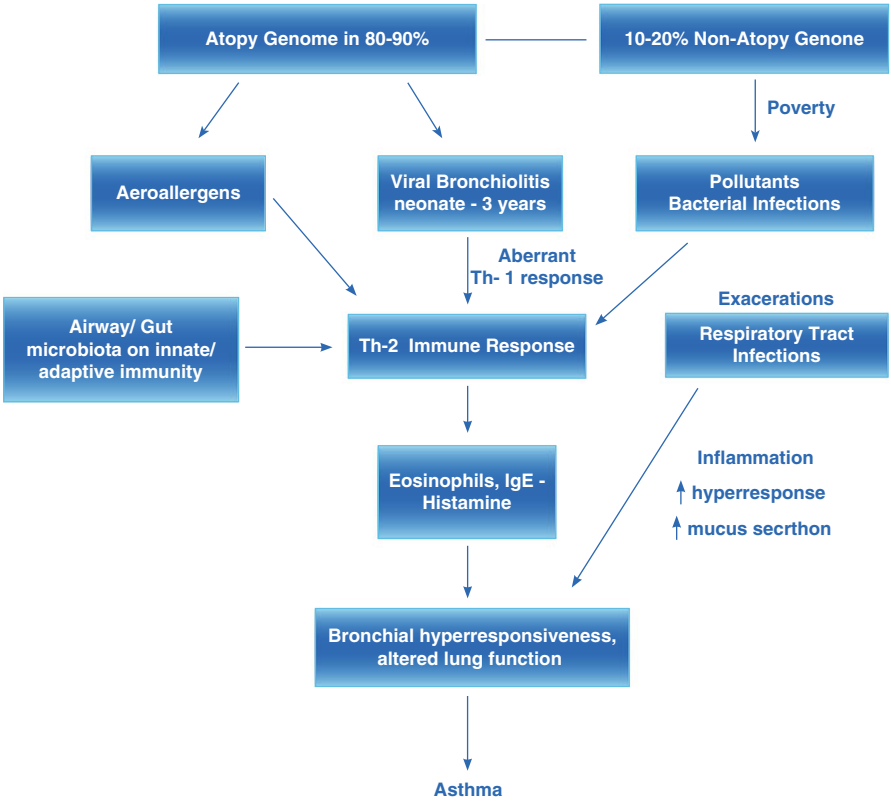


**Fig. 5.1** Paradigm of microbes in asthma pathogenesis

## 5.9 Future Directions

Further studies to elucidate the role of microbes in the causation of asthma are definitely needed and should combine the various aspects in the same high-risk birth cohorts. For example, studies starting in the antenatal period through school age should assess by modern molecular methods the environmental microbial composition, i.e., house dust, the microbiota of the maternal feces, vagina and uterine cavity at delivery, and in the newborn samples from the upper airway or hypopharynx and fecal specimens all should be tested together; plus documentation of subsequent lower respiratory tract infections up to 3 years of age. Then analyze and correlate these various factors with development of school-age asthma.

Before embarking on any large randomized controlled trials in early childhood of the value of probiotics in prevention of asthma several elements should be met. There should be agreement among a committee of experts on the composition of the probiotics chosen to be studied. Pilot studies should be performed on a small sample of subjects to determine whether or not the probiotic supplements produced the desired changes in the gut or upper airway microbiota composition. Endpoints should include skin test reactivity to aeroallergens, atopic manifestations such as eczema, development of wheezing and asthma, and the persistence in subsequent years in school.

Another prospective, longitudinal study in high-risk birth cohorts that is warranted is the assessment of diet on fecal and oro-pharyngeal microbiota, levels of vitamin D and respiratory infections over 8–10 years or more, and later development of asthma in a large sample of children.

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